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THE ORIGIN OF VARIATIONS

SYMPOSIUM AT THE THIRTY-NINTH ANNUAL MEETING OF THE AMERICAN SOCIETY OF NATURALISTS, TORONTO, DECEMBER 29, 1921

VARIATION IN UNIPARENTAL REPRODUCTION

PROFESSOR H. S. JENNINGS

THE JOHNS HOPKINS UNIVERSITY

DARWINISM left the origin of variations the unsolved problem. Give us inherited variations, it said, and we can explain adaptation, by natural selection. But this was the omission of 99 per cent., if not 100 per cent., of the problem of evolution. Are we in better case to-day? Has the experimental study of genetics given us some solid knowledge of the origin, the causes, of variation? Have we learned that the obvious differences observable everywhere among individuals are the foundations of evolution? Or that they are not? Are slight quantitative fluctuations the material out of which evolution is made? Have we discovered that extensive saltations are the steps in evolution? Or that less extensive mutations, qualitative or chemical changes, that may be minute or large, are that by which evolution is constituted? Do we know the origin of such saltations, mutations? Have we found that the present constitution of the organism predetermines in some way the course of further change; or that an *élan vital* is driving the organism to unfold in a definite way, like a flower; that evolution is orthogenesis? Do we comprehend the nature and causes of such a push to unfold, and of the direction in which it tends? Have we found perhaps, as at least one investigator maintains,

that it is mixing of stocks, hybridization, that is the origin of organic diversity? Or do we know that the physical and chemical conditions of the environment produce changes that are inherited and give us evolution? Or finally, do several or all of these methods of action concur?

Such are the questions, I take it, on which we hope for light in the discussion this afternoon, and in the discussion of orthogenesis before the American Society of Zoologists, and of the species concept before the Botanical Section of the American Association.

The lines of attack on the problem of variation are as manifold as are the questions to be answered. The basic idea in that attack whose results I shall try to summarize is this: In the reproduction from two parents familiar to us in higher animals and plants, there is a mixing of different stocks, a formation of great numbers of diverse groups of the hereditary materials, with consequent production of a great variety of diverse offspring from a given pair of parents. This is the chief cause of the differences everywhere observable among individuals: differences formerly classed as variations and considered the material of evolutionary change. But such kaleidoscopic regrouping of materials, the units of which are not changing, has no obvious relation with evolutionary variation; in the next generation a new grouping of the same material occurs, and so on indefinitely. If there likewise occur progressive evolutionary changes, these are so lost, so hidden, in the multitude of kaleidoscopic recombinations that they can not be distinguished; the literature of evolution is filled with confusion due to this difficulty.

Therefore the idea suggests itself: Why not avoid at once all this, by studying evolutionary changes in those organisms where no mixing of stocks is occurring; where there is no kaleidoscopic regrouping of the hereditary materials? There are organisms that reproduce from a single parent, with no shifting or recombination of the germ plasm; in these, actual changes that persist from

generation to generation, such as evolution requires, should lie open before us, unconfused. We should see evolution occurring, as we see water flowing.

This plain, simple and optimistic maxim has, I fear, like many another such, not proved so illuminating as its promise. But led by it, investigators set themselves at the study of the passage of generations, with selection and propagation of individuals showing diversities, in these creatures where seemingly all lasting change from parent to offspring must be evolutionary. Their hope was to see evolution occurring. And what did they see? I need not review the details; Johannsen, Barber, Hanel, the present writer, Lashley, Agar, and others, followed for long periods the passage of generations in many different organisms during uniparental reproduction.

Their report, after years of work, was astonishingly simple and clear. As to the origin of hereditary variations, it resembled the famous chapter on the Snakes of Ireland. It summed itself, in effect, in the succinct, sufficient, exhaustive proposition that there is no inherited variation; hence no origin of such variation. There is nothing to find out about it, for it doesn't occur. The individuals produced in uniparental reproduction may indeed differ, but these diversities are transitory effects of environmental differences; they are not inherited. All the descendants of a single individual are genetically and hereditarily alike; they form in effect a set of identical twins. And from this it could be concluded that in biparental reproduction *all* the observed diversities are due to the kaleidoscopic regrouping of hereditary materials; nothing to evolutionary change.

Outcries—objurgations and acclamations—greeted these propositions. Some reviled them for their manifest absurdity, others acclaimed them for their obvious truth and the clarification they wrought. Opponents tried to disprove them by investigating the matter themselves; their evidence strengthened the propositions they

had thought to overthrow; who came to scoff remained to mourn.

Such was, in the gross, the upshot of the first phase of the study of uniparental inheritance; of perhaps the first ten years. But the matter could not rest here. This work cleared the ground. It showed that 99 per cent. or more of what had been called variation had nothing to do with evolutionary change—a conclusion which Mendelian study was reaching independently. Now it remained to accept that fact, to take a new hold, to grapple with the more difficult question: Is there yet an infinitesimal residuum of evolutionary change? If we select the most favorable organisms, and study them in most minute detail for sufficiently long series of generations, shall we indeed find that there are no persistent variations whatever? Such is the work that has in this field occupied, with redoubled intensity, the last ten years. What are the results of this second phase of the work?

Some of the workers devoted themselves to observational breeding work on the passage of many generations, accompanied by selection; others attempted to modify the inherited characters by physical and chemical agents. In the observational search for persisting alterations, with the attempt to accumulate their results by selection, we find, first, that many of the organisms studied have as yet defied all attempts to find any inherited variations. Such is the report of Ewing on his extended work with aphids; such is the case with the fungi studied by Brierly (1920). Such is the case with most of the strains of the infusorian *Paramecium,* studied in detail for long periods by many different observers. Only in certain deformed strains, and possibly in one or two other instances, has the occurrence of persisting variation been observed in animals living under the usual conditions. Such is the case with the great majority of the strains of the Cladocera studied with such extraordinary thoroughness for long periods by Banta (1921); out of 16 strains to which selection was applied for many generations, all but one

gave on the whole negative results; they did not change. Some of the investigators still insist that this is indeed the outcome of all this work; that all cases seeming to give other results are for one reason or another deceptive; that no hereditary variations occur; that evolutionary change has not been observed in this sort of reproduction—and presumably therefore in no other sort. Thus, for example, argue Brierly (1919), and, in effect, Victor Jollos (1921).

On the other hand, in some of the organisms studied, visible changes persisting from generation to generation of uniparental reproduction have been observed. Even in the first period of this sort of work, extremely rare "mutations" were reported by Barber in his work on bacteria, an apparent single one by Lashley in *Hydra;* a "bud variation" or two by Johannsen; and other isolated cases occurred. In the second period of the work, as a matter of observational fact, whatever the interpretation, it is certain that in the lowest Rhizopoda: in *Difflugia,* in *Centropyxis,* in *Arcella;* in the infusorian *Stylonychia,* and in certain abnormal strains of *Paramecium,* as studied in our laboratory at the Johns Hopkins University, there arise in uniparental reproduction, changes affecting both physiological and structural characters; changes that may be very slight, or of great extent; that are passed on to later generations in uniparental reproduction. By selection and breeding of the changed individuals, stocks are isolated which differ persistently from the stock with which the work of breeding began. In this way might well arise the diverse biotypes found in nature to occur within a species, in these organisms. Something similar was found by Stout in the propagation of certain plants by cuttings.

Again, among the 16 strains of Cladocera, subjected by Banta to selection for a physiological characteristic, one, and only one, showed persisting alterations, accumulated by the selective process, so that from the single strain, two continuously diverse strains were produced. Jollos

too has observed a few cases in which strains of *Paramecium* became differentiated in ways that could hardly be considered the result of environmental action. Doubtless some other cases might be collected. Here then we seem to have what we were searching for; here at last is something solid; here by our presuppositions we have evolution evolving; we have *seen* it! But as with so many of the seeming solid things of science—so these became sicklied o'er with a pale cast of thought, of doubt, of speculation. What, it is asked, is the cause, the fundamental nature, of these persistent changes? And are they indeed of a sort to be considered steps in evolution?

And when we look closely, the observational and selectional work has given us little information on these points. In *Arcella* Hegner found that certain of the inherited structural changes are mere results of increase or decrease in number of nuclei, brought about in a simple manner. But most of the changes in the lower organisms studied can not be accounted for in this way. The work of Erdmann (1920) indicates that certain persistent changes occur in *Paramecium* as a result of the periodic nuclear reorganization called endomixis. These would perhaps have only a significance similar to that of the recombinations occurring in biparental inheritance. It is a favorite speculative idea with opposing speculators that most or all of the persisting changes we have mentioned arise through irregularities in nuclear division, and hence are of little evolutionary significance, but this is thus far a mere possibility, without solid base; as the Germans say, it floats in the air. Another speculative notion is that the changes lack permanence; that if followed for a sufficiently great number of generations there would be reversion to the original condition. Whether this doubt can ever be resolved by observation can not be predicted; it depends perhaps on the number of generations demanded by the doubters.

In the attempts to modify inherited characters by physical and chemical agents, more positive evidence as

to the cause of variation has perhaps resulted. Can we not, it is asked, by subjecting the hereditary material to chemicals, to physical agents, alter it, as we can alter practically everything else in nature? Of course we can; it is easy. But when we alter it we usually kill it, or prevent it from developing; our task is like that consummation devoutly to be wished, of killing the pathogenic bacteria in a man—which is easy—but it also kills the man! Have we succeeded in so altering the germ plasm, without killing it, that it now develops differently, and transmits the diversity to its progeny?

It is easy by altering the chemical and physical conditions to change tremendously the development and characteristics of these creatures, and that without stopping life and reproduction. But in the infinitely greater proportion of cases such changes have no inherited effect; so soon as these particular conditions are removed, the progeny go back at once to the usual constitution. Such has been the result of extensive experiments of my own in modifying *Paramecium* with chemicals; and of Noyes in modifying Rotifera. Startling transformations of form, structure and function are readily produced and kept up for generations, but disappear when the offspring are reared under normal conditions. Once in our work the task seemed accomplished. After many generations of treatment with alcohol, *Paramecium* yielded monstrosities and deformities, analogous to those Stockard obtained by the same method in guinea pigs, and these deformities were transmitted after removal from alcohol, for generation after generation. This was stirring; all the energy of the laboratory was devoted to following the monstrous stock through long periods, leaving the formulation of pedigrees till time permitted. But when this could be done it appeared that all these abnormal individuals came from one single ancestor, out of the hundreds with which the experiment began; the rest had all returned at once to normal. We know that such hereditarily abnormal stocks occur at times in *Paramecium*,

produced in some frequency by an agency which takes the matter at once out of the field with which I am dealing—by the recombinations occurring at conjugation, at biparental reproduction. Our monstrous stock may have come from such an individual, included by accident in the experiment. Our spirit-stirring results faded into nothingness—a type of what has so often happened in promising work in the inheritance of environmental effects, of what will probably often happen again.

Other workers have been more successful. In the bacteria, if we can accept the accounts given by many investigators, and well summarized, for example, in Adami's "Medical Contributions to the Theory of Evolution," environmental conditions frequently alter, in an adaptive way, the persisting characteristics of the stocks, differentiating a single race into several. The difficulties of certainly working with unmixed strains is very great in these minute creatures, a fact which leads many students of experimental evolution to reject generalizations based on these organisms. Further, the extraordinary work of Löhnis (1921), recently published by the National Academy, tends, if substantiated, to so completely upset all supposed knowledge of life history in the bacteria that it will be best to omit these from consideration until the air is cleared. For similar reasons, and from considerations of space, I will not speak of the work on pathogenic Protozoa.

Turning then to those larger organisms that are isolated with as much ease as are guinea pigs, Middleton has found that differences of vigor and of rate of reproduction are produced by subjection of infusoria for long periods to diverse temperatures, and are perpetuated, after equalizing the temperatures, from generation to generation for long periods, and through the process of conjugation. At this meeting he has reported similar results produced by subjection to diverse chemicals. How far this is comparable to change of other characteristics than reproductive vigor we do not know.

Victor Jollos (1921) has just published in this field work which must make a deep impression on the study of experimental evolution, work which gives us more positive results than have before been achieved. By experimentation extending over years he has, by subjection for long periods of time, altered the resistance of the infusorian *Paramecium* to certain chemicals, and to heat. After removal of the causative agent these physiological changes are passed on from generation to generation of uniparental reproduction, for longer or shorter periods. Of extreme interest is the fact that longer subjection to the altering agent causes longer persistence after the agent is removed. The induced changes lasted in some cases for hundreds of generations, not yielding at the periodic nuclear reorganizations known as endomixis. But the acquired resistance in practically all cases finally disappeared if the organisms were continued sufficiently long in the normal conditions. Subjection to frequently varied environment hastened the disappearance of the persisting effect; and it usually disappeared at once when there occurred the profound reorganization accompanying conjugation and biparental reproduction. But in some cases, as in Middleton's results, the acquired resistance lasted through conjugation; even through several cycles of conjugation. But in all cases in which it was clear that he was dealing with resistance acquired through subjection to chemical or physical agents, it finally disappeared, after hundreds of generations, if the organisms were kept sufficiently long in an environment lacking the causative agent. Jollos is from this inclined to draw the conclusion that the changes are not comparable to the (assumedly) permanent differences that separate genotypes or species, and hence that they do not indicate a method by which such permanent differences may arise.

Here emerges an obvious logical difficulty involved in all work on the production of inherited change through environmental action. If we succeed in producing such

change, it is clear that the character altered was not a permanent one. And if after long re-subjection to the original environment the induced change disappears, it is equally clear that the new character was no more permanent than the original one. If we now assume that there are other characters that *are* permanent, not alterable by environmental action, of course we can obtain no light on these by changing those characters that can be changed. To me it appears that we have no right to assume, at the present stage in the game, that any such absolutely permanent characters exist. If this be true, then the production of changes persisting through many generations of uniparental, and even of biparental, reproduction, with the further fact that the greater the number of generations the altering agent has acted, the greater the number of generations the change persists, seems of the greatest interest. It perhaps would, if action of the environmental agent continued sufficiently long, lead to production of inherited characteristics that are as permanent as any such characters are. It is certainly, as Jollos agrees, capable of producing such diversity of biotypes as we find within a species; and it might perhaps, if the results of diverse agents are cumulative, produce any of the inherited diversities found in organisms. This is the most promising lead that we have found in the study of uniparental production.[1]

In sum, the study of variation in uniparental reproduction yields the following: The germinal or genotypic constitution in most organisms is extremely stable; in many stocks it changes not at all, so far as observation goes. To alter it by physical or chemical agents is usually to kill it. In some of the lowest organisms—rhizopods, bacteria, some infusoria—it changes with somewhat greater frequency, though still rarely. The nature of the changes, and whether they may be permanent, or must after many generations revert to the original condi-

[1] The important observations and discussions of Jollos relating to changes producible by environmental action at the time of conjugation do not fall within the compass of a discussion of uniparental reproduction.

tion, is in some dispute. In these same organisms, environmental agents may produce changes persisting through many generations of uniparental reproduction and even through biparental reproduction, the period of persistence depending partly on the number of generations through which the producing agent acted. This suggests that inherited characters as permanent as any that exist might in time be so produced. In spite of important differences of opinion among investigators, to the reviewer the facts in uniparental reproduction seem to point more toward the production of evolutionary change by the action of the environment on the germ plasm than by any of the other methods. In this respect it takes its place in that modern revival of work on the inheritance of acquired characters, of which we had so striking an example this morning, in the account of the dizzy rats and of the inheritance of their dizziness; though in the study of uniparental reproduction nothing has appeared that indicates a transfer of somatic characters to the germ.

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VARIATIONS IN DATURA DUE TO CHANGES IN CHROMOSOME NUMBER

DR. ALBERT FRANCIS BLAKESLEE

STATION FOR EXPERIMENTAL EVOLUTION, COLD SPRING HARBOR, L. I., N. Y.

Two forms with which we have recently carried on breeding experiments, the garden flower *Portulaca* and the jimson weed (*Datura Stramonium*), are strikingly different in the types of variations which they show. The *Portulaca* is procurable in a wide range of color varieties, and is apparently subject to relatively frequent mutations, both seminal and somatic, with sectorial and periclinal chimeras a common phenomenon. Sufficient breeding tests have been made to indicate that the varieties of *Portulaca* are due in large measure at least to gene mutations. In comparison with *Portulaca,* the jimson weed is relatively stable so far as gene mutations are concerned. Despite the large amount of breeding work with this species, both before and since the rediscovery of Mendel's law, only the two allelomorphic pairs of characters, purple *vs.* white flowers, and spiny *vs.* smooth capsules, have been identified aside from the pair, tall *vs.* short stature recently determined by the writer and Avery (3).

It is true that certain of our pure lines of *Datura* differ slightly from others when grown in comparable pedigrees, but the fact remains that so far as sharply contrasting Mendelian characters are concerned, the jimson weed is highly stable, while the *Portulaca* is highly mutable. Our knowledge of changes in chromosome number in other forms is not sufficient to indicate if there is any significance for the present discussion in the difference just mentioned between *Portulaca* and *Datura*.

Our interest in *Datura* began about 1910 or 1911, when the jimsons were used as demonstration material for students in genetics. In 1915 we found our first mutant which we called the Globe from the shape of its capsules.

The capsules of normal plants are ovate and the edges of the leaves somewhat toothed. Globe plants, on the contrary, have depressed capsules and broader leaves with a more entire margin (cf. 3, figs. 7 and 9). Figure 1 shows

FIG. 1. Young plants in 3-inch pots. The normal $2n$ plant is in the middle, the $(2n+1)$ Globe on the right, and the $(2n+2)$ Globe on the left.

young plants beginning to flower. In the center is a normal and on the right a Globe. The leaves of the latter are broader and more closely massed together. In the plant on the left, the Globe characters are more strongly developed. This plant represents an extreme type of the Globe mutant, and has been called the Round-leaf Globe. It is of considerable genetic interest and will be discussed later. It was at first thought that the Globe might be a tetraploid type like the Gigas *Œnothera* but a preliminary cytological investigation showed that such was not the case.

A peculiarity in the inheritance of the Globe (1, table 3) was found to be that the Globe complex is transmitted to only about one fourth of its offspring when a Globe parent is selfed; that about the same proportion of one fourth Globes only appears in the offspring when the Globe parent is crossed with pollen from a normal plant; and that the mutant character is transmitted to only a slight

extent or not at all through the pollen—to less than 2 per cent. in a large series of crosses.

The next mutant found was Cocklebur (3, fig. 11) named from the resemblance of its fruits to those of the cocklebur weed. The plant is weak and lopping and the leaves narrow and twisted.

The Poinsettia mutant (3, fig. 14) was named from a fancied resemblance of its long clustered leaves to the hothouse plant of that name. The Poinsettia is of especial interest, since this mutant was found to give curious ratios when heterozygous for color factors.

As our eyes became better trained, other mutants were added to the list, largely through the keen discrimination of Mr. Avery and Mr. Farnham, until we now have 12 main mutants with some varieties, all of which transmit their mutant characters essentially in the same way in which the Globe complex was found to be transmitted.

In addition we had a mutant which, unlike the 12 types just mentioned, was found to breed true, and since it is practically impossible to obtain crosses between it and the normal form from which it arose, it was called "New Species" (3, fig. 15). The capsules are somewhat spherical and the leaves broad, although in a race of the same type later discovered the leaves are not greatly different from the normals. Heterozygous plants of the "N. S." sometimes gave curious ratios in their offspring.

Such was the situation up to the spring of 1920, when we were fortunate in securing the cooperation of Mr. Belling in a study of the nuclear condition of our mutants. On the basis of his work we are able to make the classification of types shown in Fig. 2. In the individual figures—which of course are highly diagrammatic—the chromosomal constitution of somatic cells is represented. We have not attempted to represent the size differences determined by Mr. Belling and pictured in our paper in the morning session.[1] A word of explanation of terms is desirable. The terms diploid, triploid and tetraploid are already current to indicate a balanced condition in which each chromosomal set (we can not say chromosomal

[1] To be published shortly in the AMERICAN NATURALIST.

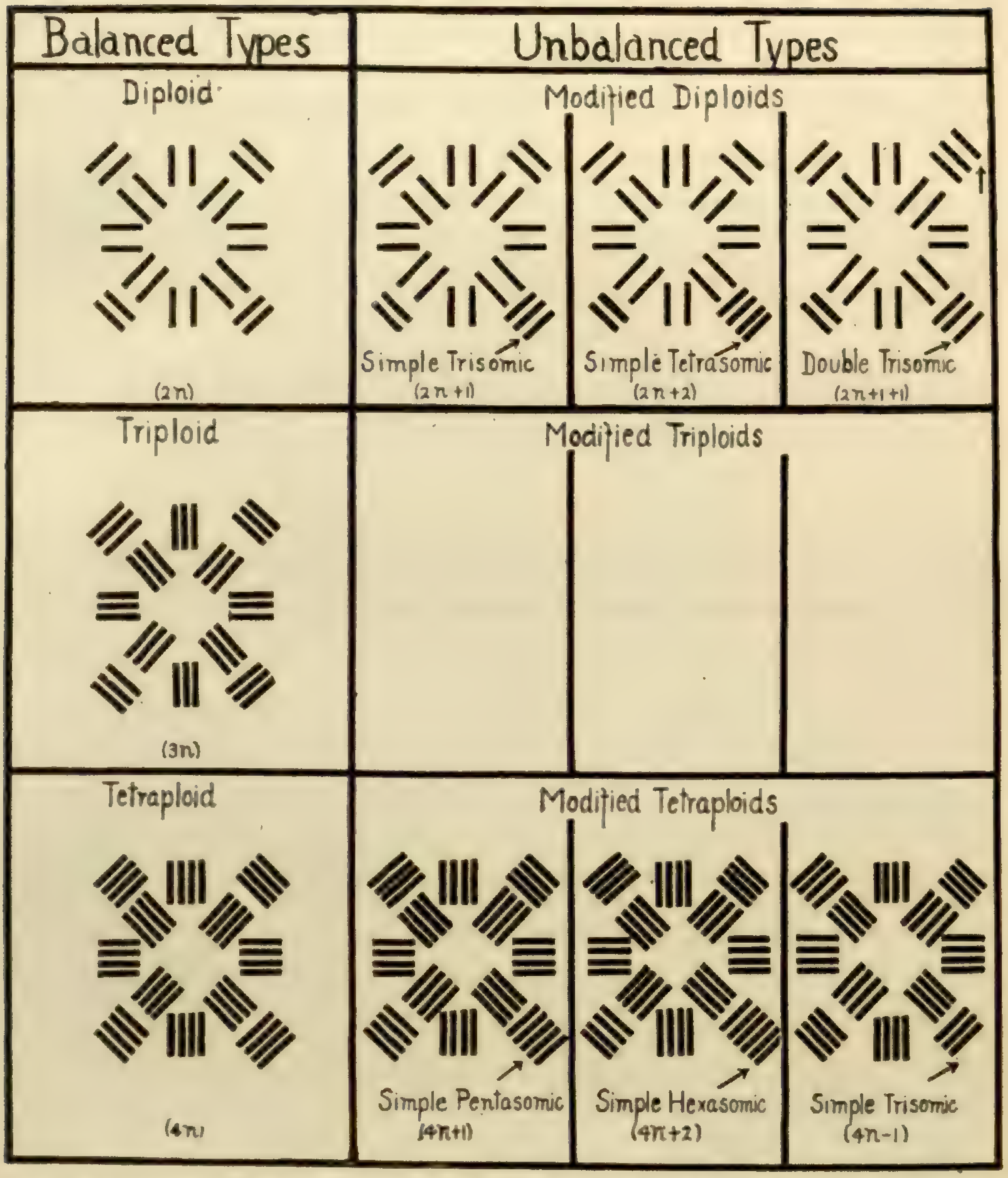

FIG. 2. Diagrams illustrating the chromosomal types already found in *Datura*.

pairs when there are more than 2 in a set) has respectively 2, 3, or 4 chromosomes. I have suggested (2) the terms disome, to indicate a set of 2 chromosomes, trisome a set of 3, and tetrasome a set of 4, etc., with the adjectives disomic, trisomic, tetrasomic, etc. Such terms may be found useful, but it seems impossible to devise a simple terminology that will adequately describe even the chromosomal irregularities at present known in *Drosophila* and *Datura*. Accordingly, after considerable discussion with Dr. Bridges, we have agreed upon a set of formulæ which is illustrated in the diagram and which we shall use in our present papers.

Of the balanced forms there are even-balanced or stable, and odd-balanced or unstable types. In the even-balanced diploid, which is the normal jimson weed, the two chromosomes in each set go to opposite poles by the ordinary process of disomic reduction, and the plants breed true for chromosome number. Partly for the same reason, the even-balanced tetraploid, which is our "New Species," breeds essentially true. The triploid, on the other hand, is odd-balanced and therefore unstable, since in the trisomic disjunction in each set two of the three chromosomes go to the one pole and one to the other, the process taking place at random. Through the operation of chance, therefore, gametes of different chromosomal number will be formed, and simple and double mutants as well as diploids will occur in the offspring. The relation may be seen from the pollen of the three balanced types under the same magnification (Fig. 3), where the photograph at the left (*a*) shows a field of pollen from a diploid; that at the right, (*c*) with larger grains, pollen from a tetraploid; while that above (*b*) shows pollen from a triploid. Pollen from a triploid is not only characterized by a large proportion of empty grains, but also by a great diversity in the size of the grains brought about by the differences in the number of chromosomes which they contain.

The upper left-hand figure of the unbalanced types (Fig. 2) has one extra chromosome in the lower right-hand set, indicated by the arrow, giving 1 trisome, and 11 disomes in this nucleus, and its formula may be written $(2n+1)$. Such a simple mutant is the Globe—simple because only one set is affected. If another set has the extra chromosome—say the set on the right—instead of the one with the arrow, this extra chromosome would cause the plant to assume the characters of, say, the Cocklebur mutant. It is obvious that since there are 12 sets in *Datura* and each set may have an extra chromosome, there are 12 mutants with the formula $(2n+1)$ theoretically possible. Through the process of disjunction in these 12 mutants, half of the gametes should contain the extra chromosome,

and half should not. Differential mortality, affecting adversely zygotes with the extra chromosome, prevents the expected equality of $(2n)$ and $(2n+1)$ individuals in the offspring from test crosses with diploids.

FIG. 3. Photomicrographs of pollen grains: (*a*) from a diploid *Datura;* (*b*) from a triploid; (*c*) from a tetraploid. The magnification is indicated by the scale, each division of which equals 0.10 mm.

The 12 mutants under discussion may best be represented in a single figure by their capsules. In Figure 4 we have capsules of the 12 simple trisomic mutants viewed from the ovate side, each one of which represents the addition of a single extra chromosome presumably in a different set. There is the Globe with depressed capsules and stocky spines; the large long-spined Poinsettia; the narrow short-spined Cocklebur; the slender-spined Ilex;

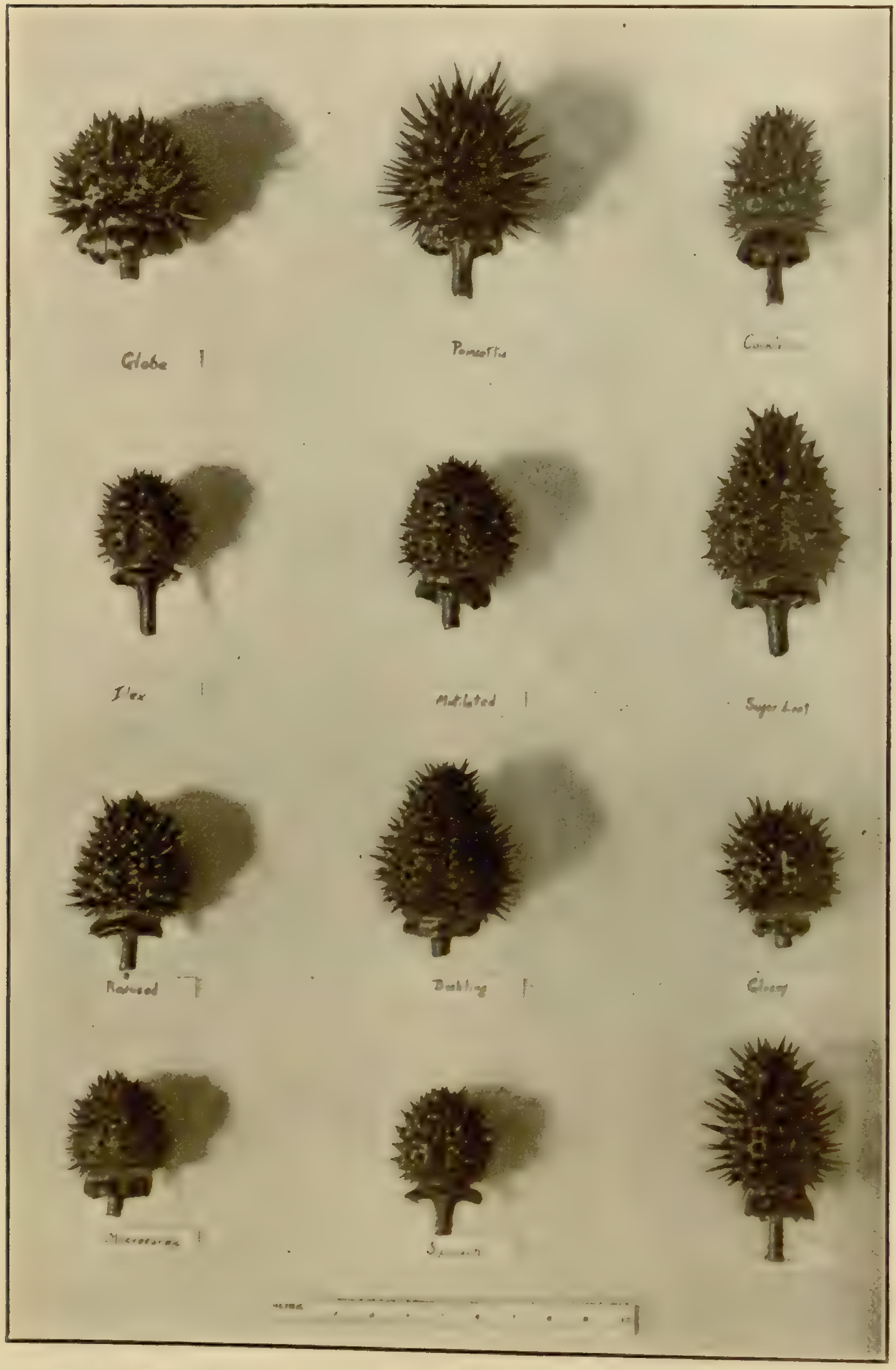

FIG. 4. Photographs of capsules of 12 mutants of *Datura* viewed from the ovate side.

the Mutilated, usually mutilated with a diseased blotch; the short-spined Sugarloaf; the shiny capsule of Glossy, etc., with lastly the narrow, long-spined Wedge. I have

provisionally called these mutants the 12 apostles. Certain of the 12 have varieties which may be called acolytes, and perhaps some of these in the figure may be reduced from the rank of apostles to that of acolytes when other forms are discovered.

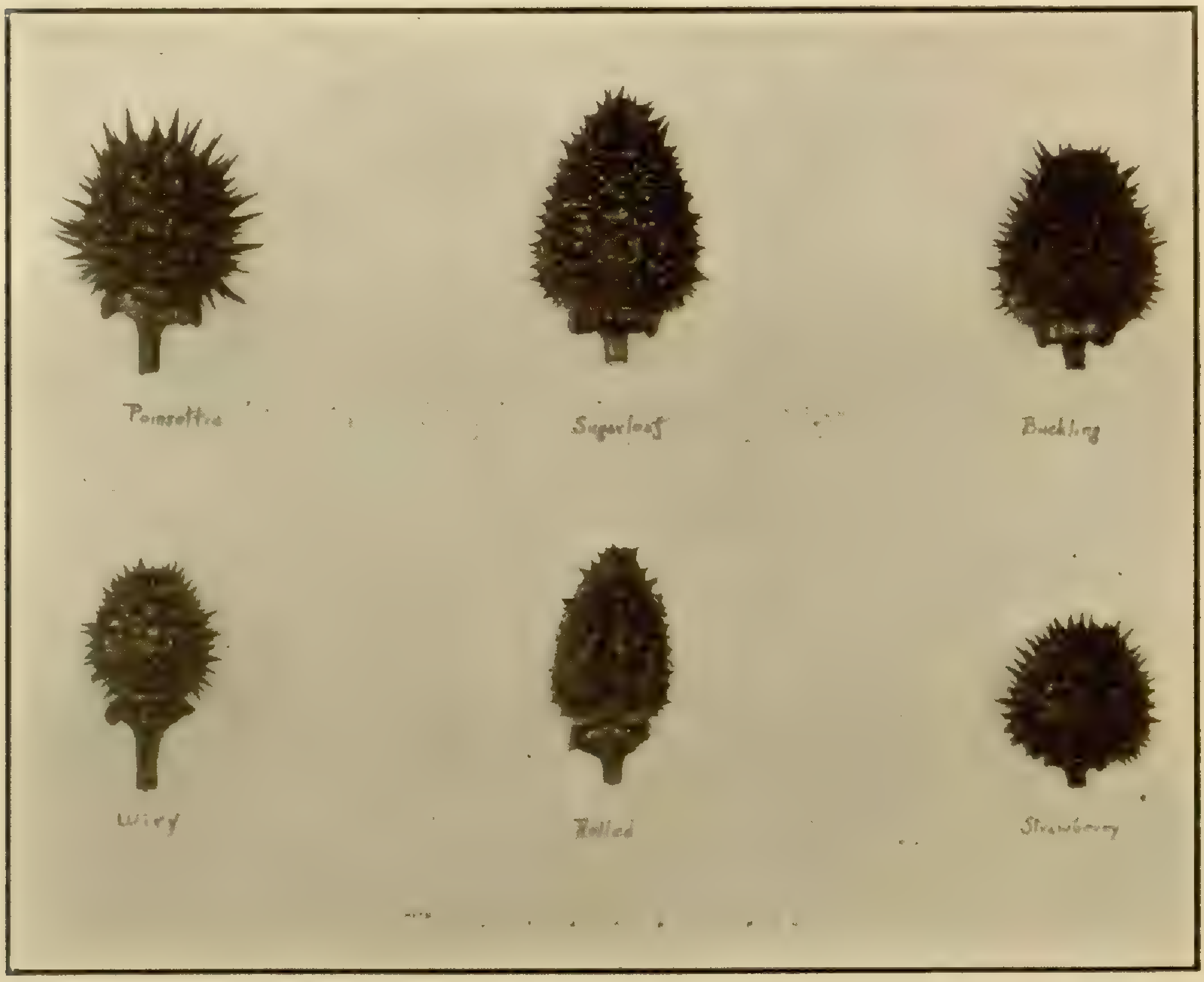

FIG. 5. Capsules representing 3 pairs of mutants. Those in the lower row are believed to represent varieties or "acolytes" of the respective types represented above.

In Figure 5, the mutants from which the capsules in the lower row were taken have been provisionally classed as acolytes of their respective apostles represented above. The evidence is best in regard to the mutants Wiry and Poinsettia which form the pair at the left in the figure. They both contain a single extra chromosome of approximately the same size, and in both cases this extra chromosome is shown, by peculiar color ratios in their offspring, to be in the set which carries the factors for purple and white flower color. The fact that though perfectly distinct they are yet similar in appearance, and the fact that one has not infrequently given rise to the other in our

cultures, is a line of argument applicable not alone to the pair Wiry and Poinsettia. It also leads us to consider Rolled an acolyte of Sugarloaf, and Strawberry an acolyte of Buckling. The possibility of acolytes being caused by modifying Mendelian factors is being investigated.

I have said that the Poinsettia mutant gave curious color ratios in its offspring (4 and 2, table 2). The evidence seems conclusive that Poinsettia has its extra chromosome in the set which carries the factors for purple and white flower color. A heterozygous Poinsettia may have one dose or two doses of the dominant purple flower color. The offspring of Poinsettia (like those of the Globe), it will be remembered, are part normals and part mutants. If a Poinsettia parent is duplex for purple, its normal offspring show 8 purples to 1 white, while its Poinsettia offspring are all purples. If the Poinsettia parent is simplex for purple the ratio for the normal offspring is 5 purples to 4 whites, and for Poinsettia offspring is 7 purples to 2 whites. The back crosses are also distinctive. By similar reasoning we believe the Cocklebur mutant has its extra chromosome in the set which carries genes for presence or absence of spines on the capsules.

The evidence is especially good for Poinsettia, since the color classes can be recognized in the seedpan. Using a Poinsettia which arose in a purple line from Washington, D. C., we crossed it with a white line of similar appearance also from Washington, and, without going outside of these two lines, have synthesized Poinsettias of all the possible combinations of color factors and have made nearly all the possible combinations of crosses between them. The results with the Washington lines are in accord with what would be expected from a random assortment of 3 chromosomes in the set containing the purple-white color factors. In a certain group of Poinsettias simplex for purple in which the 2 chromosomes bearing the white factor might have been brought in, so far as we knew, either from the white Washington stock, or from a distinct white line from Erfurt, Germany, the color ratios in the offspring of some parents were according to calcu-

lation, but from other parents the whites were approximately 6 times as frequent as would be expected. Later experiments seem to indicate: that we get the definite excess in white offspring from simplex parents when both the "white" chromosomes come from the German line; that we get the Poinsettia ratios typical of random assortment when the two white chromosomes come from the Washington whites; and that we get both of the two types of ratios from different individual F_2 parents when we make up an F_1 Poinsettia containing both a Washington white, and a German white chromosome. It is apparent that the peculiarity must be attributed to the German chromosomes. The question is receiving further experimental investigation but our provisional hypothesis to account for the difference in the ratios is that for some reason in trisomic disjunction the German white chromosomes go to opposite poles rather than to the same pole 6 times as frequently as the laws of random assortment would dictate.

Let us return to our diagrams in Fig. 2. Of the modified diploids we may have 2 extra chromosomes in a single set forming a simple mutant of the formula $(2n+2)$. An example is the round-leaf Globe (fig. 1) already mentioned.

If two different sets are affected each with a single extra chromosome we have a double mutant with the formula $(2n+1+1)$. Of the 66 different double trisomic mutants theoretically possible, we have a considerable number now under cultivation. As an example, the double mutant Globe-Reduced is shown in Fig. 6. At the top is a capsule of a normal diploid with its chromosomal diagram. At the left is a capsule of the Globe, and at the right a capsule of Reduced. Their diagrams indicate that the two mutants have different sets affected. The plant represented by the capsules below, from the appearance of its leaves as well as from that of its fruit, is undoubtedly a double mutant with the two sets affected as indicated in the diagram below. If the Globe-Reduced behaves like other double mutants we have bred, its off-

spring should contain normal diploids, both the Globe and the Reduced mutants, as well as the double mutant, Globe-Reduced, roughly in the proportion of 6 : 2 : 2 : 1.

Triploids (fig. 2) have been discussed in this morning's session. Our prediction at last year's meeting has been

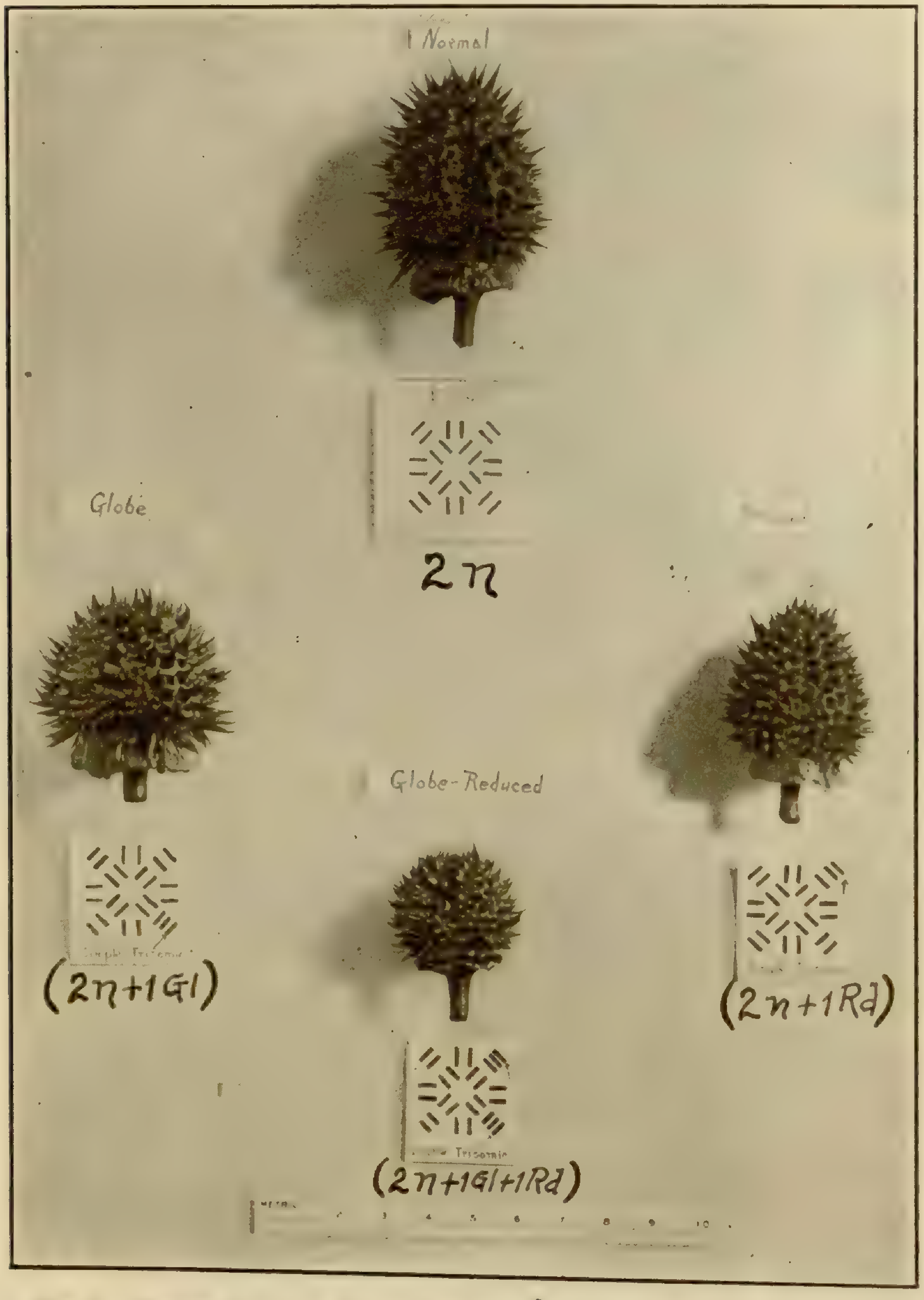

FIG. 6. Capsule of normal diploid (2*n*) above; capsule of Reduced (2*n* + 1 *Rd*) at right; capsule of Globe (2*n* + 1 *Gl*) at left; and capsule of double mutant Globe-Reduced (2*n* + 1 *Gl* + 1 *Rd*) below. Below each capsule is given its chromosomal diagram.

fulfilled and we have obtained, in the offspring of a triploid, practically the full range of $(2n+1)$ mutants as well as double mutants of the formula $(2n+1+1)$. No modified triploids have as yet been identified, but even if we found them we could not expect to be able to propagate them by seed.

Heterozygous tetraploid plants also show curious ratios, according to whether there are 1, 2, or 3 doses of the dominant factor. Duplex plants give a 35: 1 ratio when selfed and the different types in the offspring segregate in a characteristic fashion.

In the tetraploids we may have a single extra chromosome in one set making a simple $(4n+1)$ mutant, or 2 chromosomes in a set making a simple $(4n+2)$ mutant. We have two cases of a tetraploid with a deficiency in one set, producing a $(4n-1)$ mutant.

Up to the present time, except for Gregory's work on tetraploid *Primulas* (5) which was correctly interpreted by Muller (6), Mendelian research has dealt almost exclusively with disomic inheritance. Our work with the jimsons and the recent investigations of Bridges on triploid *Drosophilas* offer an opportunity for the rather novel study of trisomic, tetrasomic and pentasomic inheritance. We do not believe, however, that the jimson weed is peculiar among plants in giving rise to chromosomal mutants.

The unbalancing effect of the extra chromosomes can best be illustrated by extra chromosomes in the Globe set. The $(2n+2)$ Globe has two extra chromosomes in the Globe set and hence should show a greater divergence from normal than the Globe with only one extra chromosome. Such is the case. The simple $(2n+1)$ Globe (like other mutants of this type) is less vigorous in growth than normals. The $(2n+2)$ Globe is still less vigorous than the more common $(2n+1)$ Globe. From fig. 1 it will be seen, further, that the Globe characters in the $(2n+2)$ Globe on the left, such as broadness of leaves, fatness of bud, and density of foliage, are much further developed

than in the $(2n+1)$ Globe at the right, which has only one extra chromosome.

Photographs of capsules (Fig. 7) will further illustrate the idea of unbalance. Unfortunately the $(2n+2)$ Globe just mentioned fruits poorly and none of its capsules were available when the fruits of the other types were photographed. Later a photograph of a capsule was made to the same scale, and inserted in the proper place in the series. It will be evident that the Globe characters of relative stockiness of spines and depression of capsules are more marked in the $(2n+2)$ Globe where there are 2 extra chromosomes in the Globe set than in the $(2n+1)$ Globe on the left where there is only one extra chromosome in this particular set. Likewise in the modified tetraploids the (plus 2) Globe on the right is more Globe-like than the (plus 1) Globe beside it.

The degree of unbalance of chromosomes in the nuclei may be given a quantitative expression. Thus in the $(2n+1)$ Globe, the extra chromosome produces an excess of one over the balanced $2n$ condition. The nucleus is overbalanced by the active factors in a single Globe chromosome. This unbalance may be said to be 1 over $2n$. In a similar way the $(2n+2)$ Globe with 2 extra chromosomes has an unbalance of 2 over $2n$. Having in mind these quantitative differences one would expect the $(4n+1)$ Globe with an unbalance of 1 over $4n$ to show a less marked expression of the Globe characters than the $(2n+1)$ Globe with an unbalance of 1 over $2n$. They are, in fact, less readily recognized in recording our pedigrees. The relation of unbalance enabled us to predict the possibility of finding $(4n+2)$ Globes with an unbalance of 2 over $4n$ which one would expect to be as distinct in appearance as $(2n+1)$ Globes with an equivalent unbalance of 1 over $2n$. The prediction has been fulfilled and we are led to expect the appearance of Globes with 3, and Globes with 4 extra chromosomes in the Globe set, if tetraploid plants can endure the extreme unbalance of 4 over $4n$, the equivalent of 2 over $2n$ obtained in the $(2n+2)$ Globe.

It must be emphasized that our quantitative expressions of unbalance hold strictly only for the chromosomal numbers in reference to a single set, and not necessarily for the somatic characters conditioned by them, although the nuclear unbalance seems to be reflected in the somatic

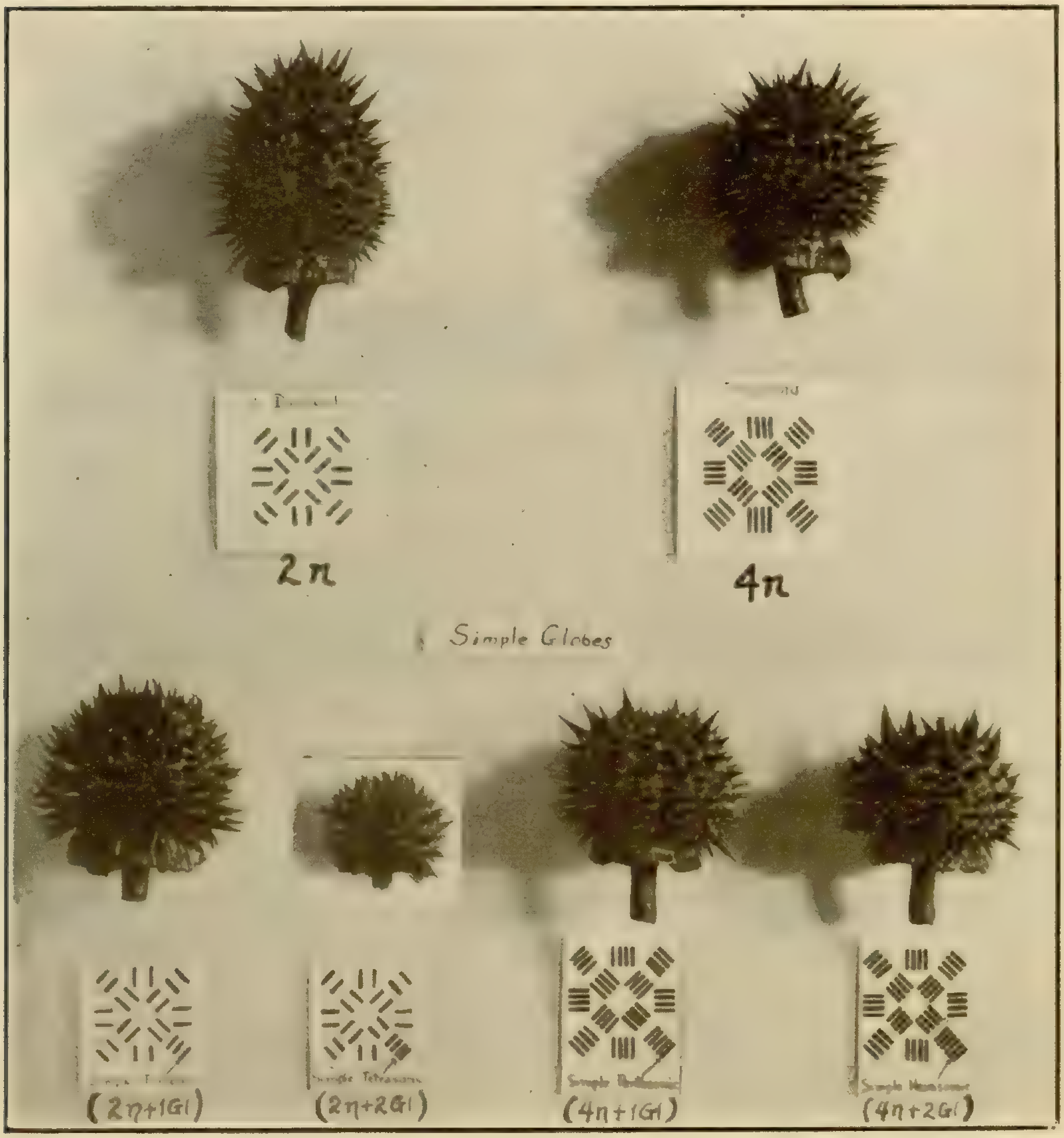

FIG. 7. Above, capsules with diagrams of a diploid (2*n*) and a tetraploid (4*n*). Below, capsules with diagrams of the different Globe mutants.

appearance, at least in the Globe series just discussed. In double mutants, moreover, somatic effects may be intensified or largely neutralized by individual genes in the two extra chromosomes, and an easy expression of the combined unbalance which they exert will therefore be impossible.

The structural characters have been taken for illustra-

tion from a particular part of a single mutant, the Globe. A more detailed study of changes in external and internal morphology brought about by the presence of specific extra chromosomes in the several mutants is being undertaken in cooperation with Dr. Sinnott.

The unbalancing effect of an extra chromosome is shown in the lessened vigor of mutant plants. Thus from Globe parents as an example of $(2n + 1)$ mutants, ordinarily only one quarter of the offspring to reach recordable size are Globes, instead of the 50 per cent. expected. Moreover, when the plants are crowded the proportion of Globes surviving is considerably lessened.

We have been discussing the unbalance as affecting the sporophytic generation. In the gametophyte, the unbalance is doubled. Thus from $(2n + 1)$ Globe plants with an unbalance of 1 over $2n$ the pollen grains with the extra chromosome have an unbalance of 1 over n. This extreme unbalance hinders their functioning and brings it about that the Globe character is transmitted to only a slight extent through the pollen (under 2 per cent. in a considerable series of crosses). It is of interest in this connection to note the results of selfing and crossing Globes of the tetraploid series. The unbalance in a $(4n + 1)$ Globe is 1 over $4n$, while the unbalance in its pollen grains which carry the extra chromosome is 1 over $2n$. Due to this lessened unbalance in comparison with pollen of $(2n + 1)$ Globes, the pollen of the $(4n + 1)$ Globe transmits the Globe character to a higher percentage of its progeny (14 per cent. in the single pedigree tested), and partially for the same reason we have obtained higher proportions of Globes in the offspring from selfing such $(4n + 1)$ Globes (a total of about 60 per cent. in a single experiment). A more specific study of the effect of extra chromosomes upon the gametophyte is being undertaken in cooperation with Dr. Buchholz.

It will not be advisable at the present stage of our investigations to discuss the possible external and internal factors which may induce the chromosomal aberrations which form the basis of our common mutations in *Datura*.

A study of the effects of radium rays undertaken in cooperation with Dr. Gager has given results which, although in an early stage of the experiment, appear suggestive in this connection. Other stimuli are being tested which appear to induce irregularities in the distribution of chromosomes to the pollen grains. It will be a matter of theoretical interest to be able to control experimentally the production of chromosomal mutations. It might also prove to be of considerable economic importance to be able to produce at will the full range of chromosomal mutants in any plants, especially in those which are propagated by vegetative means.

To us, one of the most interesting features of the *Datura* work is the possibility afforded of analyzing the influence of individual chromosomes upon both the morphology and physiology of the plant without waiting for gene mutations. Evidence is at hand which indicates that every chromosome in *Datura* carries factors which influence the expression of the so-called unit character purple pigmentation. Our work so far we believe adds evidence to the conclusion that the mature organism—plant or animal—is not a structure like a child's house of blocks, made up of separate unit characters, nor is it determined by separate and unrelated unit factors. It is rather the resultant of a whole series of interacting and more or less conflicting forces contained in the individual chromosomes.

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VARIATION DUE TO CHANGE IN THE INDIVIDUAL GENE[1]

DR. H. J. MULLER

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF TEXAS

I. THE RELATION BETWEEN THE GENES AND THE CHARACTERS OF THE ORGANISM

THE present paper will be concerned rather with problems, and the possible means of attacking them, than with the details of cases and data. The opening up of these new problems is due to the fundamental contribution which genetics has made to cell physiology within the last decade. This contribution, which has so far scarcely been assimilated by the general physiologists themselves, consists in the demonstration that, besides the ordinary proteins, carbohydrates, lipoids, and extractives, of their several types, there are present within the cell *thousands* of distinct substances—the " genes "; these genes exist as ultramicroscopic particles; their influences nevertheless permeate the entire cell, and they play a fundamental rôle in determining the nature of all cell substances, cell structures, and cell activities. Through these cell effects, in turn, the genes affect the entire organism.

It is not mere guesswork to say that the genes are ultra-microscopic bodies. For the work on *Drosophila* has not only proved that the genes are in the chromosomes, in definite positions, but it has shown that there must be hundreds of such genes within each of the larger chromosomes, although the length of these chromosomes is not over a few microns. If, then, we divide the size of the chromosome by the minimum number of its genes, we find that the latter are particles too small to give a visible image.

The chemical composition of the genes, and the formulæ of their reactions, remain as yet quite unknown. We do know, for example, that in certain cases a given

[1] Contribution No. 156.

pair of genes will determine the existence of a particular enzyme (concerned in pigment production), that another pair of genes will determine whether or not a certain agglutinin shall exist in the blood, a third pair will determine whether homogentisic acid is secreted into the urine (" alkaptonuria "), and so forth. But it would be absurd, in the third case, to conclude that on this account the gene itself consists of homogentisic acid, or any related substance, and it would be similarly absurd, therefore, to regard cases of the former kind as giving any evidence that the gene *is* an enzyme, or an agglutinin-like body. The reactions whereby the genes produce their ultimate effects are too complex for such inferences. Each of these effects, which we call a " character " of the organism, is the product of a highly complex, intricate, and delicately balanced system of reactions, caused by the interaction of countless genes, and every organic structure and activity is therefore liable to become increased, diminished, abolished, or altered in some other way, when the balance of the reaction system is disturbed by an alteration in the nature or the relative quantities of any of the component genes of the system. To return now to these genes themselves.

II. The Problem of Gene Mutability

The most distinctive characteristic of each of these ultra-microscopic particles—that characteristic whereby we identify it as a gene—is its property of self-propagation: the fact that, within the complicated environment of the cell protoplasm, it reacts in such a way as to convert some of the common surrounding material into an end-product identical in kind with the original gene itself. This action fulfills the chemist's definition of " autocatalysis "; it is what the physiologist would call " growth "; and when it passes through more than one generation it becomes " heredity." It may be observed that this reaction is in each instance a rather highly localized one, since the new material is laid down by the side of the original gene.

The fact that the genes have this autocatalytic power is in itself sufficiently striking, for they are undoubtedly complex substances, and it is difficult to understand by what strange coincidence of chemistry a gene can happen to have just that very special series of physico-chemical effects upon its surroundings which produces—of all possible end-products—just this particular one, which is identical with its own complex structure. But the most remarkable feature of the situation is not this oft-noted autocatalytic action in itself—it is the fact that, when the structure of the gene becomes changed, through some "chance variation," the catalytic property of the gene may[2] become correspondingly changed, in such a way as to leave it still *auto*catalytic. In other words, the change in gene structure—accidental though it was—has somehow resulted in a change of exactly *appropriate* nature in the catalytic reactions, so that the new reactions are now accurately adapted to produce more material just like that in the new changed gene itself. It is this paradoxical phenomenon which is implied in the expression "variation due to change in the individual gene," or, as it is often called, "mutation."

What sort of structure must the gene possess to permit it to mutate in this way? Since, through change after change in the gene, this same phenomenon persists, it is evident that it must depend upon some general feature of gene construction—common to all genes—which gives each one a *general* autocatalytic power—a "carte blanche"—to build material of whatever specific sort it itself happens to be composed of. This general principle of gene structure might, on the one hand, mean nothing more than the possession by each gene of some very simple character, such as a particular radicle or "side-chain"—alike in them all—which enables each gene to enter into combination with certain highly organized materials in the outer protoplasm, in such a way as to result in the formation, "by" the protoplasm, of more material like this gene which is in combination with it. In

[2] It is of course conceivable, and even unavoidable, that *some* types of changes do destroy the gene's autocatalytic power, and thus result in its eventual loss.

that case the gene itself would only initiate and guide the direction of the reaction. On the other hand, the extreme alternative to such a conception has been generally assumed, perhaps gratuitously, in nearly all previous theories concerning hereditary units; this postulates that the chief feature of the autocatalytic mechanism resides in the structure of the genes themselves, and that the outer protoplasm does little more than provide the building material. In either case, the question as to what the general principle of gene construction is, that permits this phenomenon of mutable autocatalysis, is the most fundamental question of genetics.

The subject of gene variation is an important one, however, not only on account of the apparent problem that is thus inherent in it, but also because this same peculiar phenomenon that it involves lies at the root of organic evolution, and hence of all the vital phenomena which have resulted from evolution. It is commonly said that evolution rests upon two foundations—inheritance and variation; but there is a subtle and important error here. Inheritance by itself leads to no change, and variation leads to no permanent change, unless the variations themselves are heritable. Thus it is not inheritance *and* variation which bring about evolution, but the inheritance *of* variation, and this in turn is due to the general principle of gene construction which causes the persistence of autocatalysis despite the alteration in structure of the gene itself. Given, now, any material or collection of materials having this one unusual characteristic, and evolution would automatically follow, for this material would, after a time, through the accumulation, competition and selective spreading of the self-propagated variations, come to differ from ordinary inorganic matter in innumerable respects, in addition to the original difference in its mode of catalysis. There would thus result a wide gap between this matter and other matter, which would keep growing wider, with the increasing complexity, diversity and so-called "adaptation" of the selected mutable material.

III. A Possible Attack through Chromosome Behavior

In thus recognizing the nature and the importance of the problem involved in gene mutability have we now entered into a *cul de sac,* or is there some way of proceeding further so as to get at the physical basis of this peculiar property of the gene? The problems of growth, variation and related processes seemed difficult enough to attack even when we thought of them as inherent in the organism as a whole or the cell as a whole—how now can we get at them when they have been driven back, to some extent at least, within the limits of an invisible particle? A gene can not effectively be ground in a mortar, or distilled in a retort, and although the physicochemical investigation of other biological substances may conceivably help us, by analogy, to understand its structure, there seems at present no method of approach along this line.

There is, however, another possible method of approach available: that is, to study the behavior of the chromosomes, as influenced by their contained genes, in their various physical reactions of segregation, crossing over, division, synapsis, etc. This may at first sight seem very remote from the problem of getting at the structural principle that allows mutability in the gene, but I am inclined to think that such studies of synaptic attraction between chromosomes may be especially enlightening in this connection, because the most remarkable thing we know about genes—besides their mutable autocatalytic power—is the highly specific attraction which like genes (or local products formed by them) show for each other. As in the case of the autocatalytic forces, so here the attractive forces of the gene are somehow exactly adjusted so as to react in relation to more material of the same complicated kind. Moreover, when the gene mutates, the forces become readjusted, so that they may now attract material of the new kind; this shows that the attractive or synaptic property of the gene, as well as its catalytic property, is not primarily dependent on its specific structure, but on some general principle of its make-up, that

causes whatever specific structure it has to be auto-attractive (and autocatalytic).

This auto-attraction is evidently a strong force, exerting an appreciable effect against the non-specific mutual repulsions of the chromosomes, over measurable microscopic distances much larger than in the case of the ordinary forces of so-called cohesion, adhesion and adsorption known to physical science. In this sense, then, the physicist has no parallel for this force. There seems, however, to be no way of escaping the conclusion that in the last analysis it must be of the same nature as these other forces which cause inorganic substances to have specific attractions for each other, according to their chemical composition. These inorganic forces, according to the newer physics, depend upon the arrangement and mode of motion of the electrons constituting the molecules, which set up electro-magnetic fields of force of specific patterns. To find the principle peculiar to the construction of the force-field pattern of genes would accordingly be requisite for solving the problem of their tremendous auto-attraction.

Now, according to Troland (1917), the growth of crystals from a solution is due to an attraction between the solid crystal and the molecules in solution caused by the similarity of their force field patterns, somewhat as similarly shaped magnets might attract each other—north to south poles—and Troland maintains that essentially the same mechanism must operate in the autocatalysis of the hereditary particles. If he is right, each different portion of the gene structure must—like a crystal—attract to itself from the protoplasm materials of a similar kind, thus moulding next to the original gene another structure with similar parts, identically arranged, which then become bound together to form another gene, a replica of the first. This does not solve the question of what the general principle of gene construction is, which permits it to retain, like a crystal, these properties of auto-attraction,[3] but if the main point is correct, that

[3] It can hardly be true, as Troland intimates, that all similar fields attract each other more than they do dissimilar fields, otherwise all substances would be autocatalytic, and, in fact, no substances would be soluble. More-

the autocatalysis is an expression of specific attractions between portions of the gene and similar protoplasmic building blocks (dependent on their force-field patterns), it is evident that the very same forces which cause the genes to grow should also cause like genes to attract each other, but much more strongly, since here all the individual attractive forces of the different parts of the gene are summated. If the two phenomena are thus really dependent on a common principle in the make-up of the gene, progress made in the study of one of them should help in the solution of the other.

Great opportunities are now open for the study of the nature of the synaptic attraction, especially through the discovery of various races having abnormal numbers of chromosomes. Here we have already the finding by Belling, that where three like chromosomes are present, the close union of any two tends to exclude their close union with the third. This is very suggestive, because the same thing is found in the cases of specific attractions between inorganic particles, that are due to their force field patterns. And through Bridges' finding of triploid *Drosophila,* the attraction phenomena can now be brought down to a definitely genic basis, by the introduction of specific genes—especially those known to influence chromosome behavior—into one of the chromosomes of a triad. The amount of influence of this gene on attraction may then be tested quantitatively, by genetic determination of the frequencies of the various possible types of segregation. By extending such studies to include the effect of various conditions of the environment—such as temperature, electrostatic stresses, etc.—in the presence of the different genetic situations, a considerable field is opened up.

This suggested connection between chromosome behavior and gene structure is as yet, however, only a possibility. It must not be forgotten that at present we can

over, if the parts of a molecule are in any kind of "solid," three dimensional formation, it would seem that those in the middle would scarcely have opportunity to exert the moulding effect above mentioned. It therefore appears that a special manner of construction must be necessary, in order that a complicated structure like a gene may exert such an effect.

not be sure that the synaptic attraction is exerted by the genes themselves rather than by local products of them, and it is also problematical whether the chief part of the mechanism of autocatalysis resides within the genes rather than in the "protoplasm." Meanwhile, the method is worth following up, simply because it is one of our few conceivable modes of approach to an all-important problem.

It may also be recalled in this connection that besides the genes in the chromosomes there is at least one similarly autocatalytic material in the chloroplastids, which likewise may become permanently changed, or else lost, as has been shown by various studies on chlorophyll inheritance. Whether this plastid substance is similar to the genes in the chromosomes we can not say, but of course it can not be seen to show synaptic attraction, and could not be studied by the method suggested above.[4]

IV. The Attack through Studies of Mutation

There is, however, another method of attack, in a sense more direct, and not open to the above criticisms. That is the method of investigating the individual gene, and the structure that permits it to change, through a study of the changes themselves that occur in it, as observed by the test of breeding and development. It was through the investigation of the *changes* in the chromosomes—caused by crossing over—that the structure of the chromosomes was analyzed into their constituent genes in line formation; it was through study of molecular changes that molecules were analyzed into atoms tied together in definite ways, and it has been finally the rather recent finding of changes in atoms and investigation of the resulting pieces, that has led us to the present analysis of atomic structure into positive and negative electrons having characteristic arrangements. Similarly, to understand the properties and possibilities of the individual gene, we must study the mutations as directly as possible, and bring the results to bear upon our problem.

[4] It may be that there are still other elements in the cell which have the nature of genes, but as no critical evidence has ever been adduced for their existence, it would be highly hazardous to postulate them.

(*a*) *The Quality and Quantity of the Change*

In spite of the fact that the drawing of inferences concerning the gene is very much hindered, in this method, on account of the remoteness of the gene-cause from its character-effect, one salient point stands out already. It is that the change is not always a mere loss of material, because clear-cut reverse mutations have been obtained in corn, *Drosophila*, *Portulaca*, and probably elsewhere. If the original mutation was a loss, the reverse must be a gain. Secondly, the mutations in many cases seem not to be quantitative at all, since the different allelomorphs formed by mutations of one original gene often fail to form a single linear series. One case, in fact, is known in which the allelomorphs even affect totally different characters: this is the case of the truncate series, in which I have found that different mutant genes at the same locus may cause either a shortening of the wing, an eruption on the thorax, a lethal effect, or any combination of two or three of these characters. In such a case we may be dealing either with changes of different types occurring in the same material or with changes (possibly quantitative changes, similar in type) occurring in different component parts of one gene. Owing to the universal applicability of the latter interpretation, even where allelomorphs do not form a linear series, it can not be categorically denied, in any individual case, that the changes may be merely quantitative changes of some *part* of the gene. If all changes were thus quantitative, even in this limited sense of a loss or gain of part of the gene, our problem of why the changed gene still seems to be autocatalytic would in the main disappear, but such a situation is excluded a priori since in that case the thousands of genes now existing could never have evolved.

Although a given gene may thus change in various ways, it is important to note that there is a strong tendency for any given gene to have its changes of a particular kind, and to mutate in one direction rather than in another. And although mutation certainly does not always consist of loss, it often gives effects that might be termed losses. In the case of the mutant genes for

bent and eyeless in the fourth chromosome of *Drosophila* it has even been proved, by Bridges, that the effects are of exactly the same kind, although of lesser intensity, than those produced by the entire loss of the chromosome in which they lie, for flies having bent or eyeless in one chromosome and lacking the homologous chromosome are even more bent, or more eyeless, than those having a homologous chromosome that also contains the gene in question. The fact that mutations are usually recessive might be taken as pointing in the same direction, since it has been found in several cases that the loss of genes—as evidenced by the absence of an entire chromosome of one pair—tends to be much more nearly recessive than dominant in its effect.

The effect of mutations in causing a loss in the characters of the organism should, however, be sharply distinguished from the question of whether the gene has undergone any loss. It is generally true that mutations are much more apt to cause an apparent loss in character than a gain, but the obvious explanation for that is, not because the gene tends to lose something, but because most characters require for proper development a nicely adjusted train of processes, and so any change in the genes—no matter whether loss, gain, substitution or rearrangement—is more likely to throw the developmental mechanism out of gear, and give a "weaker" result, than to intensify it. For this reason, too, the most frequent kind of mutation of all is the lethal, which leads to the loss of the entire organism, but we do not conclude from this that all the genes had been lost at the time of the mutation. The explanation for this tendency for most changes to be degenerative, and also for the fact that certain other kinds of changes—like that from red to pink eye in *Drosophila*—are more frequent than others—such as red to brown or green eye—lies rather in developmental mechanics than in genetics. It is because the developmental processes are more unstable in one direction than another, and easier to push "downhill" than up, and so any mutations that occur—no matter what the gene change is like—are more apt to have these *effects*

than the other *effects*. If now selection is removed in regard to any particular character, these character changes which occur more readily must accumulate, giving apparent orthogenesis, disappearance of unused organs, of unused physiological capabilities, and so forth. As we shall see later, however, the changes are not so frequent or numerous that they could ordinarily push evolution in such a direction against selection and against the immediate interests of the organism.

In regard to the magnitude of the somatic effect produced by the gene variation, the *Drosophila* results show that there the smaller character changes occur oftener than large ones. The reason for this is again probably to be found in developmental mechanics, owing to the fact that there are usually more genes slightly affecting a given character than those playing an essential rôle in its formation. The evidence proves that there are still more genes whose change does not affect the given character at all—no matter what this character may be, unless it is life itself—and this raises the question as to how many mutations are absolutely unnoticed, affecting no character, or no detectable character, to any appreciable extent at all. Certainly there must be many such mutations, judging by the frequency with which "modifying factors" arise, which produce an effect only in the presence of a special genetic complex not ordinarily present.

(*b*) *The Localization of the Change*

Certain evidence concerning the causation of mutations has also been obtained by studying the relations of their occurrence to one another. Hitherto it has nearly always been found that only one mutation has occurred at a time, restricted to a single gene in the cell. I must omit from consideration here the two interesting cases of deficiency, found by Bridges and by Mohr, in each of which it seems certain that an entire region of a chromosome, with its whole cargo of genes, changed or was lost, and also a certain peculiar case, not yet cleared up, which has recently been reported by Nilson-Ehle; these important

cases stand alone. Aside from them, there are only two instances in which two (or more) new mutant genes have been proved to have been present in the same gamete. Both of these are cases in *Drosophila*—reported by Muller and Altenburg (1921)—in which a gamete contained two new sex-linked lethals; two cases are not a greater number than was to have been expected from a random distribution of mutations, judging by the frequency with which single mutant lethals were found in the same experiments. Ordinarily, then, the event that causes the mutation is specific, affecting just one particular kind of gene of all the thousands present in the cell. That this specificity is due to a spatial limitation rather than a chemical one is shown by the fact that when the single gene changes the other one, of identical composition, located near by in the homologous chromosome of the same cell, remains unaffected. This has been proved by Emerson in corn, by Blakeslee in *Portulaca,* and I have shown there is strong evidence for it in *Drosophila.* Hence these mutations are not caused by some general pervasive influence, but are due to "accidents" occurring on a molecular scale. When the molecular or atomic motions chance to take a particular form, to which the gene is vulnerable, then the mutation occurs.

It will even be possible to determine whether the entire gene changes at once, or whether the gene consists of several molecules or particles, one of which may change at a time. This point can be settled in organisms having determinate cleavage, by studies of the distribution of the mutant character in somatically mosaic mutants. If there is a group of particles in the gene, then when one particle changes it will be distributed irregularly among the descendant cells, owing to the random orientation of the two halves of the chromosome on the mitotic spindles of succeeding divisions,[5] but if there is only one particle to

[5] This depends on the assumption that if the gene does consist of several particles, the halves of the chromosomes, at each division, receive a random sample of these particles. That is almost a necessary assumption, since a gene formed of particles each one of which was separately partitioned at division would tend not to persist as such, for the occurrence of mutation in one particle after the other would in time differentiate the gene into a number of different genes consisting of one particle each.

change, its mutation must affect all of the cells in a *bloc*, that are descended from the mutant cell.

(*c*) *The Conditions under which the Change occurs*

But the method that appears to have most scope and promise is the experimental one of investigating the conditions under which mutations occur. This requires studies of mutation frequency under various methods of handling the organisms. As yet, extremely little has been done along this line. That is because, in the past, a mutation was considered a windfall, and the expression "mutation frequency" would have seemed a contradiction in terms. To attempt to study it would have seemed as absurd as to study the conditions affecting the distribution of dollar bills on the sidewalk. You were simply fortunate if you found one. Not even controls, giving the "normal" rate of mutation—if indeed there is such a thing—were attempted.[6] Of late, however, we may say that certain very exceptional banking houses have been found, in front of which the dollars fall more frequently—in other words, specially mutable genes have been discovered, that are beginning to yield abundant data at the hands of Nilsson-Ehle, Zeleny, Emerson, Anderson and others. For some of these mutable genes the rate of change is found to be so rapid that at the end of a few decades half of the genes descended from those originally present would have become changed. After these genes have once mutated, however, their previous mutability no longer holds. In addition to this "banking house method" there are also methods, employed by Altenburg and myself, for—as it were—automatically sweeping up wide areas of the streets and sifting the collections for the valuables. By these special genetic methods of reaping mutations we have recently shown that the ordinary genes of *Drosophila*—unlike the mutable genes above—would usually require at least a thousand years—prob-

[6] Studies of "mutation frequency" had of course been made in the Œnotheras, but as we now know that these were not studies of the rate of gene change but of the frequencies of crossing over and of chromosome aberrations they may be neglected for our present purposes.

ably very much more—before half of them became changed. This puts their stability about on a par with, if not much higher than, that of atoms of radium—to use a fairly familiar analogy. Since, even in these latter experiments, many of the mutations probably occurred within a relatively few rather highly mutable genes, it is likely that most of the genes have a stability far higher than this result suggests.

The above mutation rates are mere first gleanings—we have yet to find how different conditions affect the occurrence of mutations. There had so far been only the negative findings that mutation is not confined to one sex (Muller and Altenburg, 1919; Zeleny, 1921), or to any one stage in the life cycle (Bridges, 1919; Muller, 1920; Zeleny, 1921), Zeleny's finding that bar-mutation is not influenced by recency of origin of the gene (1921), and the as yet inconclusive differences found by Altenburg and myself for mutation rate at different temperatures (1919), until at this year's meeting of the botanists Emerson announced the definite discovery of the influence of a genetic factor in corn upon the mutation rate in its allelomorph, and Anderson the finding of an influence upon mutation in this same gene, caused by developmental conditions—the mutations from white to red of the mutable gene studied occurring far more frequently in the cells of the more mature ear than in those of the younger ear. These two results at least tell us decisively that mutation is not a sacred, inviolable, unapproachable process: it may be altered. These are the first steps; the way now lies open broad for exploration.

It is true that I have left out of account here the reported findings by several investigators, of genetic variations caused by treatments with various toxic substances and with certain other unusual conditions. In most of these cases, however, the claim has not been made that actual gene changes have been caused: the results have usually not been analyzed genetically and were in fact not analyzable genetically; they could just as well be interpreted to be due to abnormalities in the distribution of genes—for instance, chromosome abnormalities like

those which Mavor has recently produced with X-rays—as to be due to actual gene mutations. But even if they were due to real genic differences, the possibility has in most cases by no means been excluded (1) that these genic differences were present in the stock to begin with, and merely became sorted out unequally, through random segregation; or (2) that other, invisible genic differences were present which, after random sorting out, themselves caused differences in mutation rate between the different lines. Certain recent results by Altenburg and myself suggest that genic differences, affecting mutation rate, may be not uncommon. To guard against either of these possibilities it would have been necessary to test the stocks out by a thorough course of inbreeding beforehand, or else to have run at least half a dozen different pairs of parallel lines of the control and treated series, and to have obtained a definite difference in the same direction between the two lines of *each* pair; otherwise it can be proved by the theory of "probable error" that the differences observed may have been a mere matter of random sampling among genic differences originally present. Accumulating large numbers of abnormal or inferior individuals by selective propagation of one or two of the treated lines—as has been done in some cases—adds nothing to the significance of the results.

At best, however, these genetically unrefined methods would be quite insensitive to mutations occurring at anything like ordinary frequency, or to such differences in mutation rate as have already been found in the analytical experiments on mutation frequency. And it seems quite possible that larger differences than these will not easily be hit upon, at least not in the early stages of our investigations, in view of the evidence that mutation is ordinarily due to an accident on an ultramicroscopic scale, rather than directly caused by influences pervading the organism. For the present, then, it appears most promising to employ organisms in which the genetic composition can be controlled and analyzed, and to use genetic methods that are sensitive enough to disclose mutations occurring in the control as well as in the treated individ-

uals. In this way relatively slight variations in mutation frequency, caused by the special treatments, can be determined, and from the conditions found to alter the mutation rate slightly we might finally work up to those which affect it most markedly. The only methods now meeting this requirement are those in which a particular mutable gene is followed, and those in which many homozygous or else genetically controlled lines can be run in parallel, either by parthenogenesis, self-fertilization, balanced lethals or other special genetic means, and later analyzed, through sexual reproduction, segregation and crossing over.

V. Other Possibilities

We can not, however, set fixed limits to the possibilities of research. We should not wish to deny that some new and unusual method may at any time be found of directly producing mutations. For example, the phenomena now being worked out by Guyer may be a case in point. There is a curious analogy between the reactions of immunity and the phenomena of heredity, in apparently fundamental respects,[7] and any results that seem to connect the two are worth following to the limit.

Finally, there is a phenomenon related to immunity, of still more striking nature, which must not be neglected by geneticists. This is the d'Hérelle phenomenon. D'Hérelle found in 1917 that the presence of dysentery bacilli in the body caused the production there of a filterable substance, emitted in the stools, which had a lethal and in fact dissolving action on the corresponding type of bacteria, if a drop of it were applied to a colony of the bacteria that were under cultivation. So far, there would be nothing to distinguish this phenomenon from im-

[7] I refer here to the remarkable specificity with which a particular complex antigen calls forth processes that construct for it an antibody that is attracted to it and fits it "like lock and key," followed by further processes that cause more and more of the antibody to be reproduced. *If* the antigen were a gene, which could be slightly altered by the cell to form the antibody that neutralized it—as some enzymes can be slightly changed by heating so that they counteract the previous active enzyme—and if this antibody-gene then became implanted in the cell so as to keep on growing, all the phenomena of immunity would be produced.

munity. But he further found that when a drop of the affected colony was applied to a second living colony, the second colony would be killed; a drop from the second would kill a third colony, and so on indefinitely. In other words, the substance, when applied to colonies of bacteria, became multiplied or increased, and could be so increased indefinitely; it was self-propagable. It fulfills, then, the definition of an autocatalytic substance, and although it may really be of very different composition and work by a totally different mechanism from the genes in the chromosomes, it also fulfills our definition of a gene.[8] But the resemblance goes further—it has been found by Gratia that the substance may, through appropriate treatments on other bacteria, become changed (so as to produce a somewhat different effect than before, and attack different bacteria) and still retain its self-propagable nature.

That two distinct kinds of substances—the d'Hérelle substances and the genes—should both possess this most remarkable property of heritable variation or "mutability," each working by a totally different mechanism, is quite conceivable, considering the complexity of protoplasm, yet it would seem a curious coincidence indeed. It would open up the possibility of two totally different kinds of life, working by different mechanisms. On the other hand, if these d'Hérelle bodies were really genes, fundamentally like our chromosome genes, they would give us an utterly new angle from which to attack the gene problem. They are filterable, to some extent isolable, can be handled in test-tubes, and their properties, as shown by their effects on the bacteria, can then be studied after treatment. It would be very rash to call these bodies genes, and yet at present we must confess that there is no distinction known between the genes and them. Hence we can not categorically deny that perhaps we may be able to grind genes in a mortar and cook them in a beaker after all. Must we geneticists become bac-

[8] D'Hérelle himself thought that the substance was a filterable virus parasitic on the bacterium, called forth by the host body. It has since been found that various bacteria each cause the production of D'Hérelle substances which are to some extent specific for the respective bacteria.

teriologists, physiological chemists and physicists, simultaneously with being zoologists and botanists? Let us hope so.

I have purposely tried to paint things in the rosiest possible colors. Actually, the work on the individual gene, and its mutation, is beset with tremendous difficulty. Such progress in it as has been made has been by minute steps and at the cost of infinite labor. Where results are thus meager, all thinking becomes almost equivalent to speculation. But we can not give up thinking on that account, and thereby give up the intellectual incentive to our work. In fact, a wide, unhampered treatment of all possibilities is, in such cases, all the more imperative, in order that we may direct these labors of ours where they have most chance to count. We must provide eyes for action.

The real trouble comes when speculation masquerades as empirical fact. For those who cry out most loudly against "theories" and "hypotheses"—whether these latter be the chromosome theory, the factorial "hypothesis," the theory of crossing over, or any other—are often the very ones most guilty of stating their results in terms that make illegitimate *implicit* assumptions, which they themselves are scarcely aware of simply because they are opposed to dragging "speculation" into the open. Thus they may be finally led into the worst blunders of all. Let us, then, frankly admit the uncertainty of many of the possibilities we have dealt with, using them as a spur to the real work.

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THE ORIGIN OF VARIATIONS IN SEXUAL AND SEX-LIMITED CHARACTERS

DR. CALVIN B. BRIDGES

Columbia University

In dealing with sex and its determination, attention has been most sharply focused upon forms with separate sexes and upon the visible differences between the chromosome groups of the two sexes. The result has been that the formulation of sex-determination has remained in terms of chromosomes, while the modern unit of determination is the gene; and also the subject of sex has been rather separated off from the main body of heredity. My discussion will be largely a process of resolving chromosomes into component genes, and showing that the conception of the nature and action of genes as gained from the study of non-sexual characters is valid in interpreting sex phenomena.

The facts of mutation and of linkage have given us the conception of a gene as a distinct chemical entity having a definite location in a particular chromosome. Each gene is essentially a factory, which is manufacturing a characteristic set of chemical products that are delivered to the common cytoplasm, and that produce development through interaction with each other and with materials from outside. But since the chemicals produced by the different genes are different, some genes will have much effect upon one character and little effect upon another, so that a relatively small proportion of the genes will be actively concerned in producing any given character. Some of these genes tend to make the character more pronounced, and others tend to make it less pronounced, so that the grade of development actually realized by each particular character will be determined by the equilibrium between its modifying genes. The forms into which a given character can be modified are in general quite diverse, but for the sake of simplicity we may call them all plus or minus modifications. If the effectiveness of a given plus or minus modifier is changed by mutation, the grade of the character will shift correspondingly.

We can conceive of the evolution of the sexual

characters of hermaphrodites in terms of successive simple mutations in genes. But to interpret male and female forms with observed differences in number or size of chromosomes and with sex-linked inheritance requires comparison with mutations in which the unit of change is a whole chromosome or section of chromosome instead of a single gene. Such mutations can be understood in terms of the action of component genes as follows. Linkage experiments show that the various kinds of genes are distributed pretty much at random among the various chromosomes and along each chromosome. But since the number of genes with a given tendency is relatively small, any particular small section of chromosome might not contain these genes in the same proportion as they exist in the entire complement, and still less would the normal proportion of every kind be present. The loss of a section of chromosome (a condition known as deficiency) would ordinarily remove more minus than plus modifiers (or vice-versa), and since in that case more plus than minus modifiers would remain in action, the grade of the corresponding character would be shifted in a minus direction. This is the interpretation of the fact that a deficiency may cause many character changes, the complex of altered characters being inherited as a dominant. When a whole chromosome is lost through non-disjunction, the effects are similar to those in deficiency for a section except that they are greater in degree.

The way in which genes act together in producing a character, and the relation of the balance of plus and minus modifiers to deficiency or to the absence of a chromosome may perhaps be made clearer by an analogy. Let us suppose that a man is an ardent stamp collector, and has accumulated a lot of stamps. These stamps are to represent genes, so their number may be put at 5,000 to correspond roughly to the number of genes in *Drosophila*. Among the Russian stamps, especially those of recent issue, there is a very large number of reds, but also a fair number of pinks, and even a few whites. These differences in tint correspond to the plus and minus modifiers of a certain character, namely, the redness of Russian stamps. Now the stamps of different tints are in some definite ratio, whatever that ratio is, and we will call it the

normal ratio or balance. This stamp collector carries his collection around with him, and it fills two big, coat pockets, a trousers pocket, and there are even a few in his vest pocket. But unlike most collectors this one has never taken the trouble to sort over more than a few of his stamps. Meanwhile he strings them together pretty much hit or miss. This stringing stamps together is rather disapproved of by some other stamp collectors, who think that is no way to treat stamps, and each of whom has his own favorite method of arranging them. Because of this hit or miss method of making the strings of stamps the ratio among the different grades of redness of Russian stamps is different in different parts of the strings, and so if some other ardent collector should snip off a piece of one of the strings and carry it away, the remainder of the Russian stamps might have a considerably redder tone, while at the same time the Polish stamps might become bluer. If a whole string were lost, then many of the sets of stamps might have quite different complexions.

Now I have been recently studying the effects of the loss of one of the chromosomes of *Drosophila*,[1] namely, the small round fourth-chromosome, and the phenomena offer striking parallels to those of diœcious sex, including sex-linked inheritance and sex-limited characters. Individuals having only one fourth-chromosome show a change in many characters, among which may be mentioned smaller size, smaller bristles, later hatching, poorer viability, paler body-color, darker trident pattern, shorter blunter wings, etc. Each of these differences corresponds to a character for which the fourth-chromosome was internally unbalanced, that is, for which the ratio of plus to minus modifiers was different from that of the whole group. For all of the characters in which there was an internal preponderance of plus modifiers the grade will be shifted in a minus direction by the loss of the fourth-chromosome, for example, the shorter wings and paler body-color. Likewise the characters that shift in a plus direction, as the darker trident pattern and the large eyes, are characters for which the fourth-chromosome possesses an excess of minus modifiers. In the

[1] *Proc. Nat'l Acad. of Sci.*, 7: 186–192.

male of *Drosophila* there is only one X-chromosome, though there is present a Y-chromosome that can be disregarded, since the evidence from non-disjunction of the X-chromosome shows that it has very little effect upon sex or characters. These individuals with only one X-

FIG. 1. Wild-type (2n) female, with normal chromosome group.

chromosome likewise show a complex of characters that are different from those shown by the individuals with the normal two X-chromosomes. Among these characters are gonads and genitalia of a type that we call male. The haplo-X individual is also smaller, has smaller bristles, is less viable, hatches later, and differs in other details from the 2-X type that we call female. Each of these differences likewise corresponds to a character for which the balance of the genes in the X is different from that in the group as a whole. The absence of one X leaves in action an unbalanced set of genes which produces male characters. The X-chromosome is a chromosome that is internally unbalanced by an excess of genes that we may call female-producing.

In an outcross of a haplo-IV individual to a normal, the entire complex of characters is inherited as a simple dominant and gives a 1 : 1 ratio, except that the haplo-IV's are less viable. Likewise in outcrosses of haplo-X individuals the entire complex of male characters is in-

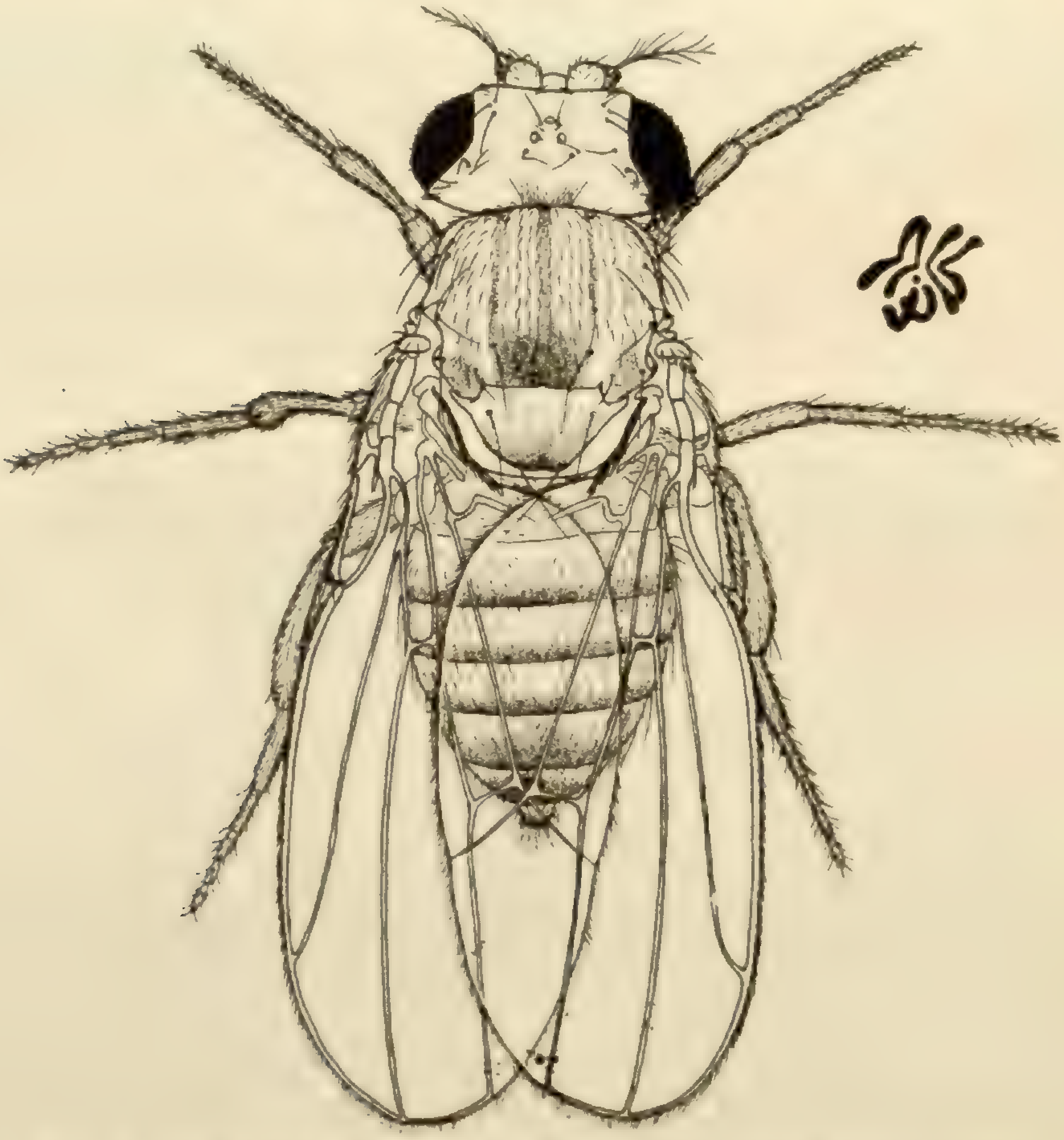

FIG. 2. Haplo-IV (2n–1 IV) female, with chromosome group.

herited as a simple dominant and gives a 1 : 1 ratio except that the haplo-X's are somewhat less viable.

When a haplo-IV individual is mated to a recessive whose gene is in the fourth-chromosome, all the haplo-fourth offspring show this recessive—a behavior that is strictly parallel to sex-linked inheritance; for if a haplo-X individual, that is, a male, is mated to a recessive whose gene is in the X, all the haplo-X offspring show this recessive.

The fourth-chromosome recessive characters present in haplo-IV individuals from the cross of a haplo-fourth to the recessive show a grade of development that is different from their grade as homozygous characters in diplo-IV's. This phenomenon is known as "exaggeration," and is interpreted as the effect of an unbalance within the

normal fourth-chromosome. With respect to a character that is exaggerated in a plus direction the fourth-chromosome has an unbalance in the minus direction. But since the whole complement is in balance, this unbalance within the fourth-chromosome is neutralized by a reciprocal unbalance in the other chromosomes. So the removal of one fourth-chromosome with its excess of minus modifiers leaves the remainder of the genes with an excess of plus modifiers, and these plus modifiers are free to work in the same direction as the recessive gene that is present, and thus to give an even greater effect than the homozygous recesive. Corresponding to these exaggerated fourth-chromosome characters there is a class of sex-linked characters that are exaggerated in the absence of one X-chromosome. These mutant characters show a different grade of development in the male from that which they show in the female. A good example is the race called eosin, in which the male has a much paler eye-color than the eosin female. These characters exaggerated by the absence of an X are called sex-limited. Some of them, like eosin, are exaggerated in a plus direction, corresponding to an excess of minus modifiers within the X-chromosome, while others, such as bobbed, are exaggerated in a minus direction. Thus bobbed, which shows scarcely at all in the males, corresponds to an excess of genes within the X tending to make bristles short, and two X-chromosomes can outweigh the genes in the autosomes that tend to make the bristles long, but one X is not enough to do so.

When haploidy for the fourth-chromosome is combined with mutants whose genes are outside the fourth-chromosome there is of course no effect corresponding to sex-linkage, but there is "exaggeration." Thus, haploidy for the fourth-chromosome exaggerates the third-chromosome mutant Hairless in a plus direction. This type of exaggeration finds its parallel in the 20 or so sex-limited mutations that are not sex-linked. These are mutations whose differential genes are in the autosomes and not in the X and which nevertheless show a different grade of development in the male from that in the female. In these cases also the modifiers of each character are of different weights in the X from the general collection,

and absence of one X leaves a surplus of genes that work in the same or in the opposite direction from that of the mutant in question.

Thus, by studying three kinds of effects, first, the character complexes that result directly, secondly, the exaggerations of the mutant characters whose genes are in the same section or chromosome as that involved in the loss, and thirdly the exaggerations of mutant characters whose genes are in other regions, we can analyse roughly the kinds and the signs of the genes that are in the region in question.

Since sexual and sex-limited characters are shown to rest on the same genetic basis, namely, a preponderance within the X of the plus or the minus modifiers of those characters, it may be questioned whether there is any real difference between these two categories. If the race of the mutant eosin were to become established in nature, a systematist would certainly include this difference in eye-color among his sexual differences. I am of the opinion that there is no difference between these two categories except that we call those sexual that are most closely connected with reproduction.

There is one striking difference between haploidy for X and haploidy for an autosome—namely, that the changes connected with haploidy for autosomes are relatively more numerous and extreme. Haploidy for the second or third autosomes probably produces changes so great as to be lethal, while haploidy for the very small fourth-chromosome produces changes comparable in extent to all those of the male aside from the reproductive organs. The proportion of sex-limited mutant characters is only about a tenth of the total, while X contains about a quarter of the genes. Since the changes in character produced by absence of an X are relatively small, the internal balance of the X must be relatively high. For a high proportion of the characters of the animal, the plus and minus modifiers in the X must be in about the same ratio as in the group as a whole.

The comparison just made between the effects of haploidy for an autosome and the effects normally present in diœcious sex shows that they have similar genic bases—namely, each is due to differences in the ratio between two

aggregates of genes; and that the X produces its characteristic effects because it contains a preponderance of genes tending to produce the characters that we call female. This point of view receives even stronger and more direct support from a study of cases in which the

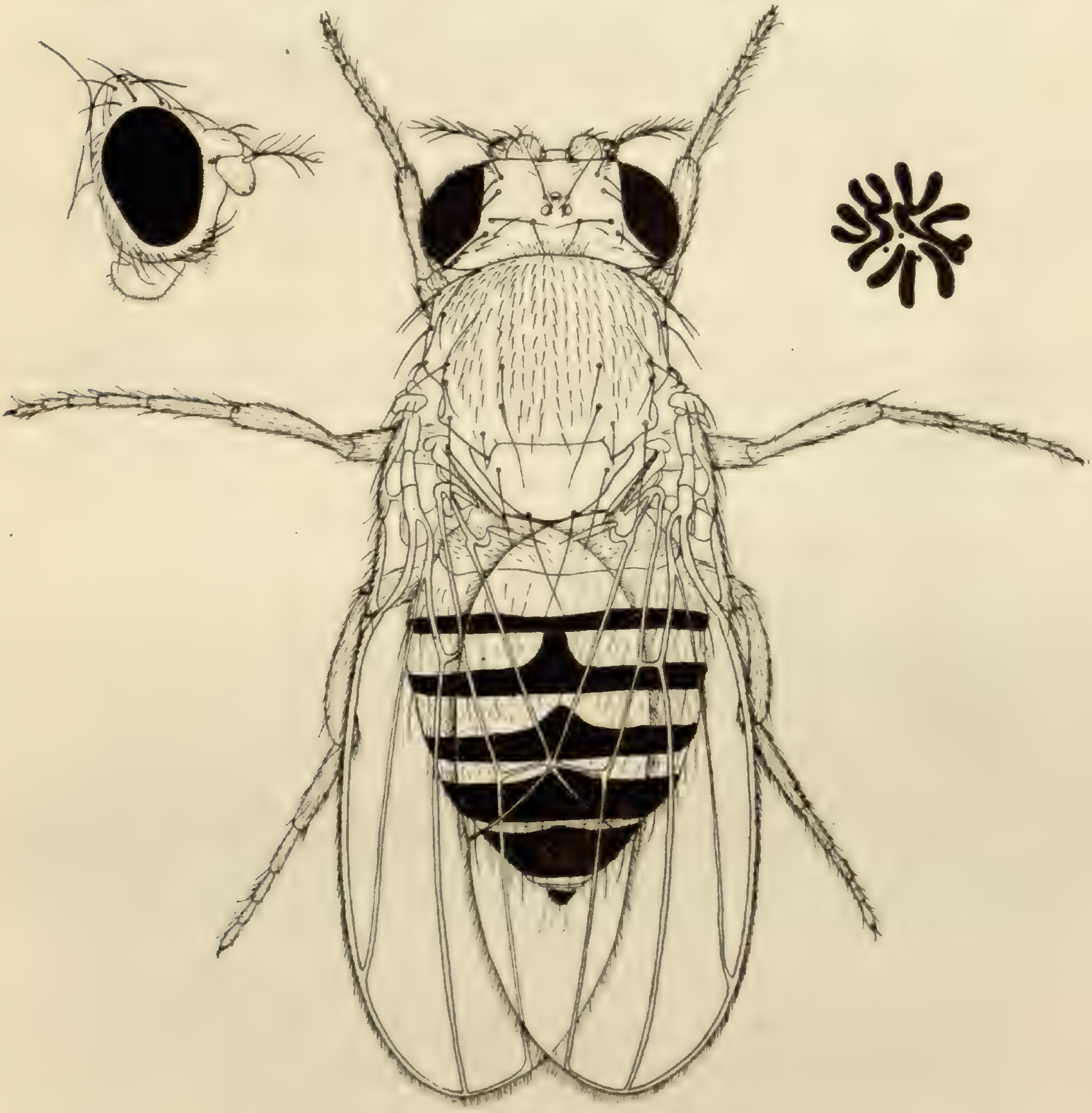

FIG. 3. Triploid (3n) female, with chromosome group.

ratio of X-chromosome to autosomes has been changed, and in which new sex relations are present. These new types of chromosome combinations and of sex take their origin in the occurrence of triploidy in *Drosophila*, for which there is full genetical and cytological proof.[2] The first point is that individuals having three full sets of chromosomes (3n) are females not to be distinguished from normal females except for slight differences in size and proportion that may well be due simply to the greater

[2] *Science*, N. S., 54: 252–254.

amount of chromatin. The nearly complete identity between the triploid and diploid forms both as to sex and as to non-sexual characters is a splendid evidence that these characters owe their grade to the ratios among the genes, for those ratios are identical in the 3n and 2n forms.

Among the offspring of triploid females are individuals that are neither males nor females but are sex-intermediates, or rather, are mixtures of male and female characters, very similar in type to the intersexes of *Lymantria*.[3] Genetical and cytological proof was obtained that these intersexes in *Drosophila* possess two X-chromosomes and three sets of autosomes. The old formulation of 2X equals ♀ is at once seen to be inadequate, for here we have individuals that have two X-chromosomes and yet are not females. They are shifted out of the female class by the presence of an extra set of autosomes, and thereby the autosomes are proved to play a positive rôle in the production of sex. Since the intersexes differ from females by the assumption of certain male characters this effect of the autosomes is due to an internal preponderance of "male-tendency" genes.

We may now formulate the sex-relations as follows: both sexes are due to the simultaneous action of two opposed sets of genes, one set tending to produce the characters called female and the other to produce the characters called male. These two sets of genes are not equally effective, for in the complement as a whole the female-tendency genes outweigh the male-tendency genes and the diploid (or triploid) form is a female. When the relative number of the female-tendency genes is lowered by the absence of one X, the male-tendency genes outweigh the female and the result is the normal haplo-X male. When the two sets of genes are acting in a ratio between these two extremes, as is the case in the ratio of *2X: 3 sets autosomes,* the result is a sex intermediate—the intersex.

The intersexes as a class can always be easily distinguished from normal males and females by reason of their large size, large coarse-textured eyes and by certain other characters such as scalloped wing-margins. Some of these characters are probably non-sexual effects of the

[3] R. Goldschmidt, *Zeit. f. ind. Abst. u. Vererb.*, 23: 1–199.

triploidy for the autosomes, others are sex-limited. Within the class of intersexes there is a very wide range of fluctuation, on the one hand to flies that are nearly female and on the other to flies that are entirely male in appearance. In an intersex of a given grade the several

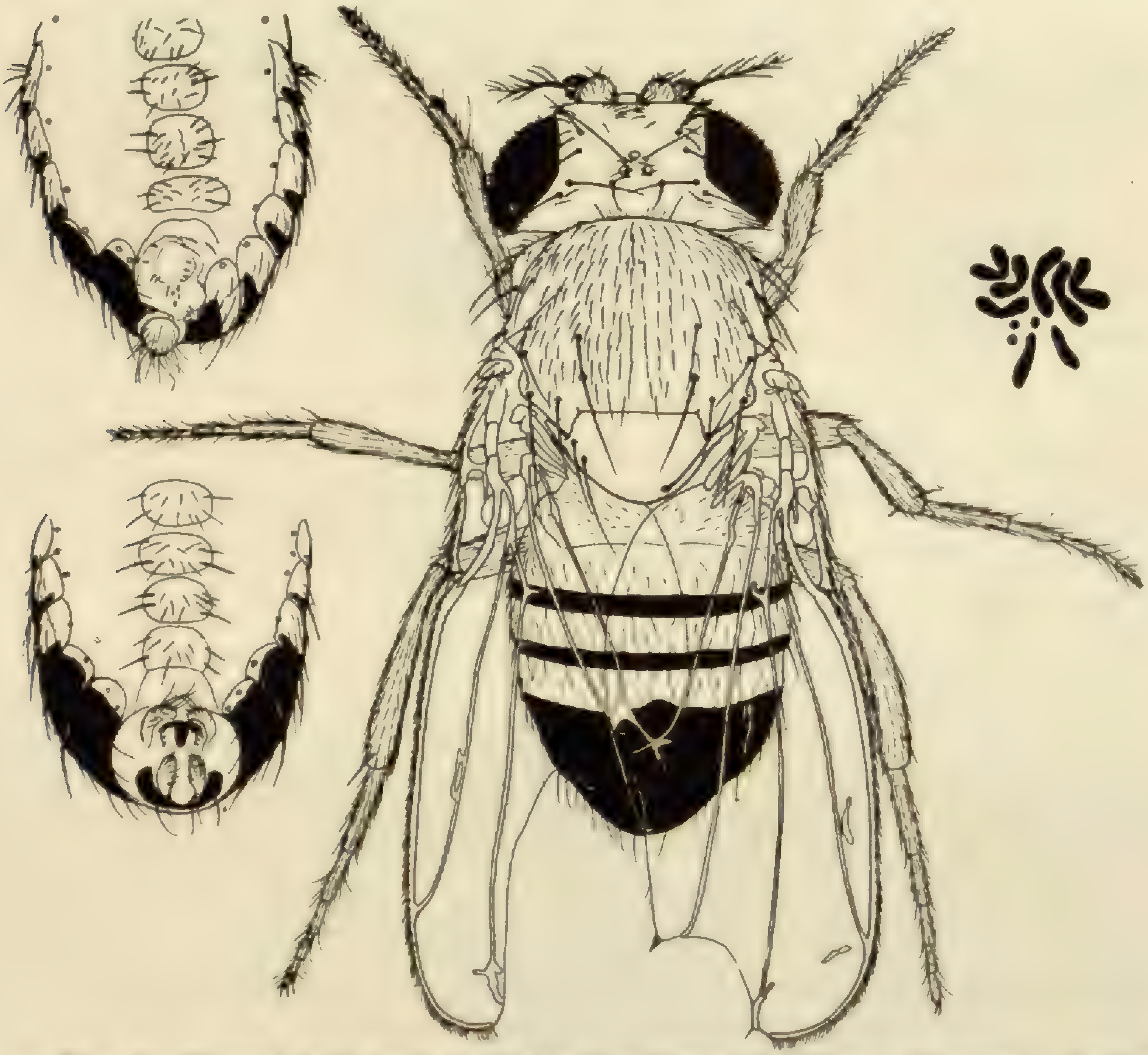

FIG. 4. Dorsal and ventral views of extreme male-type intersex. Ventral view of mid-grade intersex. Chromosome group of intersex showing 2X and 3 sets autosomes.

characters do not all present the same intermediate step between male and female, but, apparently just as in the intersexes of *Lymantria,* some characters are completely male, some completely female, while others are complex mixtures of male and female parts. When the intersexes are classified according to a system of grades, they are seen to be a bimodal class consisting of more "female-type" and more "male-type" intersexes, both of which fluctuate widely and overlap considerably.

The cytological investigation of the intersexes had shown that there are four sub-types of intersexes that differ in the presence or absence of a Y and in having three or only two fourth-chromosomes. It is possible, and there is some slight cytological and genetical evidence in support, that the male- and female-types of

intersexes correspond to the presence of three or of two fourth-chromosomes respectively.

There is another connection in which the wide fluctuations of the intersexes are interesting, namely, the action

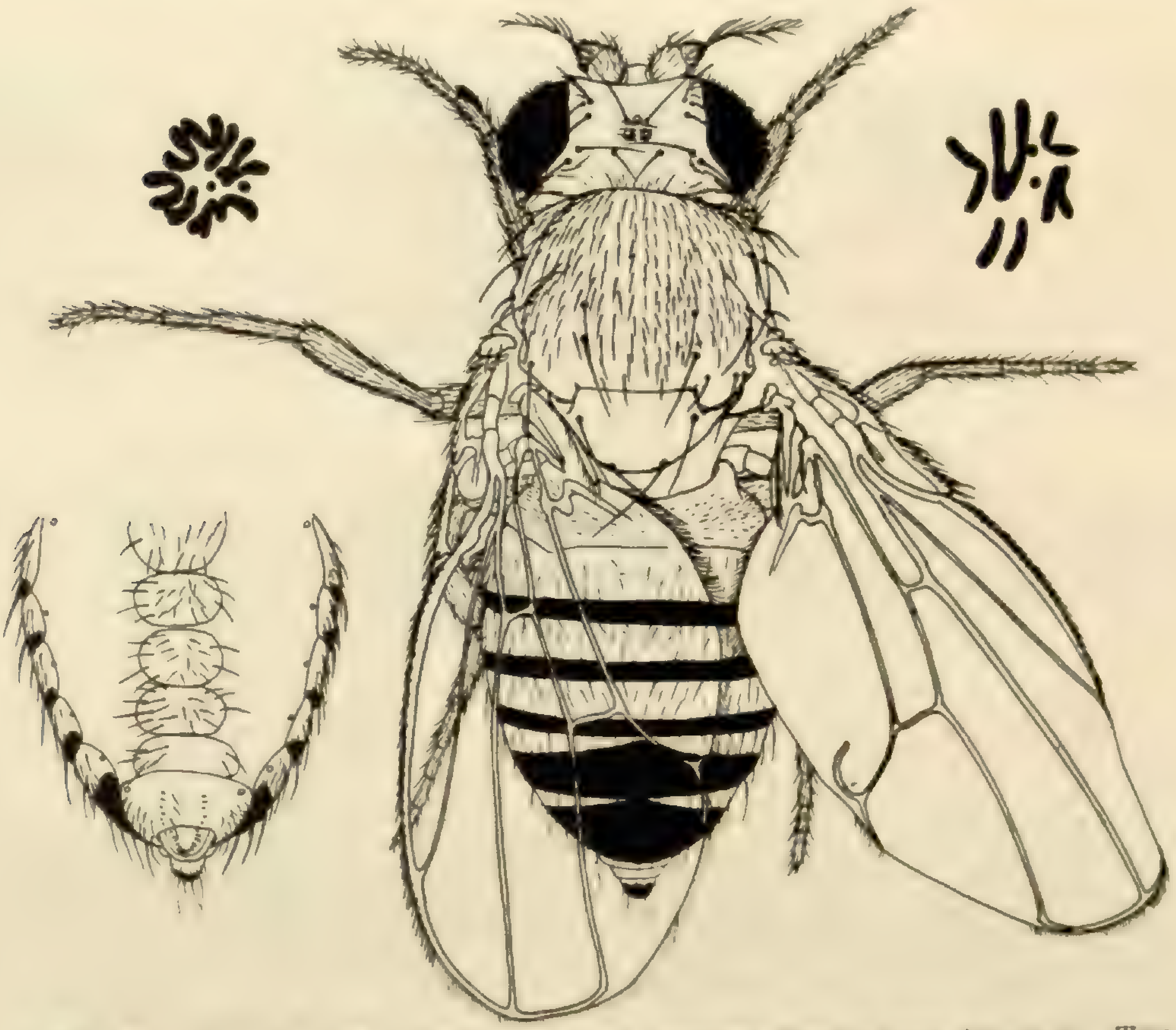

FIG. 5. Dorsal and ventral views of extreme female-type intersex. Two chromosome groups, the left with two IV-chromosomes and a Y, the right with two IV's but no Y.

of environmental factors. The slight range of fluctuation in such a character as miniature-wings in *Drosophila* probably means that there is a critical balance or ratio of plus to minus modifiers beyond which all balances give miniature, at least until the overbalance proceeds so far that a new critical ratio is passed and a new superminiature character is realized. The balance in miniature is so far beyond the critical balance that only rarely are the environmental factors strong enough to outweigh this overbalance and thus cause fluctuation. In mutants in which the overbalance is slight there will be both wide fluctuation due to environmental interference and a high susceptibility to modification by other genes, as is notoriously the case with Beaded and with Truncate.

In normal males and females there are high overbalances beyond the critical points, and consequently only slight genetical or fluctuating variations. But in the in-

tersexes these two overbalances in opposite directions cancel each other, and since the two sets of genes are now of almost exactly the same weight the point of balance is between the two critical balances. Accordingly the char-

Fig. 6. "Superfemale" (2n + X), with two chromosome groups.

acters of the intersex fluctuate widely with slight environmental differences, and fall into two modes corresponding to the slight difference in balance between two and three of the tiny fourth-chromosomes.

Relation of Sex to Chromosomes in *Drosophila melanogaster*

Sex		X-chromosomes	Sets Autosomes	Sex Index
Superfemale		3	2	1.5
Female	triploid	3	3	1
	diploid	2	2	1
Intersex	♀-type	2	3(—IV)	.67+
	♂-type	2	3	.67
Male		1	2	.5
Supermale		1	3	.33

The phenomenon of intersexuality might be expected to have a reciprocal phase—namely, supersexes. If the

intersexes result from an intermediate ratio of X to autosomes because the X has a net female tendency, then it might be expected that by increasing the ratio of X to autosomes a superfemale would be produced, and conversely, a supermale by increasing the relative number of

FIG. 7. "Supermale." No cytological evidence; genetical tests show 1X and 3 sets autosomes.

autosomes. Diploid individuals with an extra X-chromosome (2n plus X) have now been identified among the progeny of certain strains of high non-disjunction, among the offspring of triploid females and elsewhere. These flies resemble females but are very inviable and form a distinct character type. They are sterile and sections of the gonads show abnormal ovaries. These differences all result from the unbalance within the X, and are therefore of the sexual-sex-limited category. That these differences are not greater is partly due to the same high internal balance of the X that we met with in analysing males and intersexes, and is partly to be explained on the ground that for many of the characters the overbalance is not yet great enough to pass a second critical point.

Conversely, individuals with one X-chromosome and an extra set of autosomes have been identified among the offspring of triploid females. These are males distinctly different from normal males and sterile.

If there were time, it would be interesting to supplement and modify the view just presented by comparisons with the rich materials elsewhere, and perhaps to speculate as to how this machinery was evolved, and how the genes involved come to expression physiologically.

THE NATURE OF BUD VARIATIONS AS INDICATED BY THEIR MODE OF INHERITANCE[1]

PROFESSOR R. A. EMERSON

Cornell University

The title limits this account to such bud variations as have been studied critically with respect to their inheritance in sexual reproduction. The further limitation of time makes it necessary that I choose from among such studies certain cases to serve as illustrations of the several types of bud variation. I shall, therefore, attempt no complete review of the researches bearing on the problem at hand.

A survey of published accounts of bud-variation studies shows that as yet comparatively little is definitely known of the real nature of these vegetative sports. It seems not unlikely, however, that to point out some of the problems suggested by these studies and, where possible, to note modes of attack may serve the purpose of this symposium quite as well as a rehearsal of known facts and their interpretation.

As here used, the term bud variation is synonymous with vegetative as contrasted with seminal variation. The term somatic variation may also be employed to the same effect, provided it is not thereby intended to exclude cases in which the germ tract as well as the soma is involved. At the outset, however, there must be imposed on any of these terms, for the purpose of this discussion at least, the limitation that the variation involves a change in the genetic constitution of the parts affected.

The expressions somatic mutation and somatic segregation are specific terms and as such are not to be used interchangeably with the more general terms somatic, vegetative, or bud variations. Moreover, to speak of a particular vegetative variation as a case of somatic muta-

[1] Paper No. 94, Department of Plant Breeding, Cornell University, Ithaca, New York.

tion or of somatic segregation without basis from critical inheritance or cytological studies is to prejudge the nature of the observed modification.

Frequency of Somatic Variations

Attempts have been made to estimate the relative frequency of vegetative and seminal variations in plants, but little definite information has been gained. The problem is beset with grave difficulties inherent in most attempts to determine coefficients of mutability. The possibility of overlooking even prominent variations until they have once been noted, together with the readiness with which they are found after one's attention has been focused on them, will hardly be questioned by anyone who has given attention to the discovery of new variations in almost any organism. One may attempt with some assurance an estimation of the frequency of recurrence of a particular mutation, for instance, whether it appears in vegetative parts of individuals or in sexually produced progenies, but it is a hazardous undertaking to estimate the frequency of variations in general. Until some one can devise a scheme for estimating the frequency of bud variations as Muller has done for determining mutation frequencies in *Drosophila*, little progress can be looked for other than through investigations of the somatic mutation or segregation of specific genes.

The problem of the relative frequency of occurrence of somatic and gametic variations meets the further difficulty that it is often impossible to determine the ontogenetic stage at which particular variations have arisen—a fact that has been noted for plants by various writers (deVries, 1910; Emerson, 1913; East, 1917). Both Bridges (1919) and Muller (1920) have discussed this problem from the standpoint of studies of particular mutations in *Drosophila*. The prevalent opinion that variations arise in the gametes or at about the time of their formation may have come in part from a belief that aberrant chromosome behavior is most likely to occur at the time of the reduction division. It seems likely, however, that the situation has been confused by failure to realize

that recessive mutations—the most frequent kind—can not be expressed in the individual in which they occur except when the dominant allelomorph is simplex, while such mutations may appear in a later generation of sexually produced progeny (East, 1917).

Somatic Mutation of Genes

Several cases of vegetative variation in plants have been studied with sufficient thoroughness to leave little doubt that they are mutations in the strict sense, involving the modification of particular genes. Most of them are concerned with variegated color patterns of flowers, leaves, or fruits, and they are more or less regularly recurrent, a fact that makes them especially well suited to quantitative studies, for it is obvious that a quantitative study can be made only of variations that occur with considerable frequency. For the most part also these somatic mutations are dominant to the type from which they spring, appearing frequently in material homozygous for their recessive allelomorphs, facts that exclude the possibility of their being due to any sort of somatic segregation of unlike genes. Blakeslee's (1920) case of a somatic variation in *Portulaca* is one of the few examples not involving variegation. Other cases have been reported by Baur (1918).

One of the earliest cases of somatic mutation was reported by deVries in variegated flowers of *Antirrhinum*. Though the work was done prior to the rediscovery of Mendelism and not discussed from the standpoint of recent genetic interpretation, there is little doubt, as I have noted elsewhere (Emerson, 1913), that the results can best be interpreted as due to a somatic gene mutation.

Correns's (1910) results with respect to the occurrence and behavior in inheritance of green-leaved variations on variegated-leaved *Mirabilis* and of self-colored flowers on variegated flowered strains of the same species were among the first to be subjected to critical genetic analysis. The behavior in inheritance of green branches of variegated *Mirabilis* shows this vegetative variation to be a simple dominant mutation affecting ordinarily only one of the duplex recessive allelomorphs. A mutated branch

is, therefore, as truly a heterozygote as if it had arisen through hybridization of green and variegated strains.

Self-colored branches on variegated-flowered plants of *Mirabilis* usually do not transmit the self-color character to their seed progenies in greater percentages than do variegated-flowered branches of the same plants. They are thought by Correns to be fundamentally of the same nature as the green branches of variegated-leaved plants, their failure to transmit the self-color character being due presumably to the accident that the mutation occurs in epidermal cells from which no gametes arise. The frequent occurrence of self-colored plants in seed progenies both of self-colored and of variegated flowers is considered evidence of their origin as vegetative rather than as gametic mutations, their failure of expression in the soma being thought due to their origin in sub-epidermal cells in which these flower colors do not develop.

Studies of variations in variegated pericarp of maize by myself (Emerson, 1914, 1917) and by Anderson, Eyster, and Demerec,[2] involve practically the same results as those so far reported in investigations of other species and afford in addition quantitative data on certain aspects of the somatic-mutation problem not included in other investigations. The genes for variegated pericarp have been shown to belong to a comparatively large series of multiple allelomorphs including those for colorlessness (white seeds), self color of different intensities, and certain definite color patterns of both the pericarp of the seeds and the glumes and paleæ of the cobs. Variegation is known to be a simple recessive to self color and a dominant to white.

Self-colored seeds whether occurring singly or in groups in variegated ears produce progenies consisting of approximately 50 per cent. self-colored ears, the other 50 per cent. being either all variegated or all white depending on whether the parent was homozygous variegated, *V V*, or heterozygous variegated, *V W*, from a previous cross with white. Seeds that are less than wholly self colored throw a correspondingly smaller per cent. of

[2] Unpublished data by W. H. Eyster and E. G. Anderson, and by E. G. Anderson and M. Demerec.

self-colored ears. Self-colored seeds thus produced have, so far as tested, proved to be heterozygous for self color, behaving in later generations exactly as if produced by crosses of self-colored with variegated or with white races.

Certain cultures of self-colored maize produce a few variegated seeds. Such seeds have been observed only on ears that are heterozygous from previous crosses with variegated strains, *S V*, or with white strains, *S W*, never from ears that are homozygous for self color, *S S*. From such variegated seeds, new variegated races have been produced.

These facts are regarded as indicating (1) that the occurrence of self-colored or partly self-colored seeds on variegated ears is due to somatic mutations of the recessive variegation gene to the dominant self-color allelomorph; (2) that only one of the two variegation genes of homozygous variegated maize mutates at a given time; (3) that it is always the variegation gene, never the white one, of heterozygous material that mutates; (4) that the occurrence of variegated seeds on otherwise self-colored ears is due to reverse mutations from the dominant self-color gene to the recessive variegation allelomorph; and (5) that only one of the duplex genes of self-color strains so mutates at any one time, for otherwise there would remain no dominant self-color gene to prevent the expression of the mutation as variegated seeds in homozygous self-colored material.

Another type of somatic variation, quite distinct from the self-color mutations discussed above and often termed dark-crown variation, also occurs frequently in variegated maize pericarp (Emerson, 1917). It is quite as striking in appearance as the self-color mutation, but is not inherited, the progenies of the aberrant seeds being in no way different from those of the normal seeds of the same ears. Microscopic examination of dark-crown and of self-color seeds indicates that in the former the epidermis alone is colored while in the latter the epidermis alone remains colorless. The conclusion seems warranted, therefore, that the two types of variation are fundamentally the same, both being true gene mutations, and

that the non-inheritance of the dark-crown type is due to the accident that it occurs in epidermal tissue outside the germ tract.

Recent investigations of variegated maize by Eyster and Anderson have established the fact that somatic mutations affecting small areas occur much more frequently than those affecting large areas. Since a mutation arising in a single cell late in development obviously could not affect so large an area as one originating earlier, it follows that mutations in variegated maize occur with increasing frequency in the later stages of ontogeny. It is true, as pointed out by Muller (1920), that given a constant rate of mutation throughout all stages of ontogeny and granting that one cell is as likely as another to mutate, mutations should appear more frequently in the later stages of development because of the fact that there are then many more cells in which mutations may arise. But Eyster and Anderson have found that the increase in the frequency of occurrence of mutations during the progress of development is accelerated far beyond expectation based on the increase in number of cells.

This behavior is strongly suggestive of a progressive acceleration in the mutability of the variegation gene as development proceeds. It is much too early to say whether this progressive change, if such it be, is inherent in the organization of the gene itself, as suggested by Anderson and Demerec, or whether it is a response to progressive changes in physiological and environmental relations. Perhaps the assumption of an equal chance of mutation as between any two cells is without sufficient warrant. Possibly there is a time element to be taken into account, as noted by Muller (1920). As cell division becomes progressively retarded in the late growth stages, may not each cell be exposed for an increasingly longer period of time to the chance of mutation? Perhaps it may be possible to test this assumption in favorable material by a comparison of the frequency of mutation in the very early slow-growth, the later rapid-growth, and the final slow-growth periods of the life cycle; but the relatively few cells present in the very early growth period seems likely to place serious limitations on the

practicability of such a test. An observation of possible importance in connection with the question of a time element in mutation and with the problem of environmental and physiological influences is that made by Eyster and Anderson concerning the greater frequency of the non-heritable (epidermal) mutations than of the heritable (sub-epidermal) ones in variegated pericarp of maize.

I have recently obtained results bearing on another phase of the somatic-mutation problem as related to variegated maize pericarp, namely, the relative frequency of mutation of homozygous, *V V*, and of heterozygous, *V W*, material. It has been shown above that the *W* gene for colorless (white) pericarp does not mutate, so far as known, when paired either with itself, *W W*, with the variegation gene, *V W*, or with the self-color gene, *S W*. It will be recalled further that only one of the two homologous genes in homozygous variegated, *V V*, material mutates at any one time. If it could be assumed that the mutability of either allelomorph is uninfluenced by the presence of the other, it should follow that somatic mutations will occur with approximately twice the frequency in homozygous, *V V*, as in heterozygous, *V W*, material. But this expectation has not been realized. On the contrary, both heritable (self-color) and non-heritable (dark-crown) mutations have appeared throughout all my cultures with somewhat greater frequency in heterozygous than in homozygous variegated ears. The difference has been especially pronounced in very light variegated strains, where mutations have appeared about two and one half times as often in heterozygous as in homozygous material. Even if mutations appeared with equal frequency in heterozygous and in homozygous ears, the simplex gene of the former must have a mutability of about twice that of either of the duplex genes of the latter. In the very light variegated strains, therefore, a simplex gene must have a mutability of about five times that of a duplex gene.

What appears to be a similar result in *Mirabilis* has been reported by Correns (1903, 1904). Crosses of a supposedly pure white race with several self-colored pink yellow, and pale yellow races resulted in every case in

plants with strongly red-striped flowers and with numerous self red flowers or even whole branches of such flowers. Intercrosses of the pink and yellow races gave only self-colored progeny, from which fact it was concluded that the white-flowered race carried a latent factor for striping. It was later discovered that about three per cent. of the flowers of the white race showed minute flecks of red. It was evidently an extremely light, variegated race, rarely if ever throwing somatic self-color mutations when the variegation gene was duplex (homozygous material) but producing such mutations with considerable frequency when that gene was simplex (heterozygous material). Correns concluded that red variegation of *Mirabilis* flowers is a character that, with self-fertilization or inbreeding, remains almost completely latent, but which, through the entrance of foreign germ plasms, is brought to full expression.

If the mutability of a gene can be increased through the influence of some modifying factor or factors brought into combination with it by crossing, as suggested by Correns, it should be possible to discover crosses that would not produce the effects so far observed in *Zea* and *Mirabilis*. While the problem deserves much more study from this viewpoint, it seems unlikely that results with maize can be explained on any such basis, unless the postulated modifying factor is the allelomorph of the variegation gene or some factor very closely linked with it. It must be noted in this connection that the comparison in maize was made between homozygous and heterozygous variegated ears of the same F_2 progenies grown from self-pollinated F_1 heterozygotes—a circumstance that would afford abundant opportunity for recombinations of independently inherited modifying factors. That the differences in mutability noted in maize may be due to differences in the interaction of like as contrasted with that of unlike allelomorphs, as suggested by Anderson and Demerec, is a somewhat novel conception worth careful consideration if means can be devised for subjecting it to a crucial test.

Before the topic of somatic mutation is dismissed, it should be noted that the phenomenon is not limited to

plants. Among animals, *Drosophila* (Morgan and Bridges, 1919) has furnished several examples of undoubted somatic mutation resulting in mosaic individuals other than gynandromorphs.

SOMATIC SEGREGATION

Bud variations have probably been ascribed to somatic segregation more frequently than to any one other cause. Perhaps the opinion commonly held that bud variations occur more frequently in hybrids than in other material and the long known fact that seed-grown offspring of hybrids exhibit segregation, is chiefly responsible for this usage. It is, of course, possible that most vegetative variations are of this nature, but the fact that the individuals in which they arise are frequently found to be heterozygous for the genes concerned is no conclusive evidence that segregation is involved. Mutations also, as noted by several writers, are most likely to appear in heterozygous material because most of them are recessive and the unmutated dominant allelomorphs prevent their expression in the individuals in which they originate if the latter are homozygous.

Chromosome Elimination.—The best examples of somatic segregation that have been subjected to critical genetic analysis are afforded by the work with *Drosophila.* It has been shown by Morgan and Bridges (1919) that, of the relatively numerous gynandromorphs which have appeared in the course of investigations with *Drosophila,* nearly all have resulted from the elimination of the sex chromosome at some early cleavage division. If a fertilized egg starts as a female, XX, and one X chromosome is eliminated at an early segmentation that part of the individual developing from the cell that receives but one X chromosome should be male, XO, while the remaining part should be female, XX.

The evidence in support of this view was obtained from crosses the parents of which had different sex-linked and different autosomal characters, that is, characters whose genes are carried by the sex chromosomes and by the autosomes, respectively. The male, as well as the female,

side of gynandromorphs appearing in such crosses exhibited all the dominant autosomal characters whether they came from the maternal or the paternal parent. When the mother had a recessive, mutant gene in one of her autosomes and the father had its dominant, normal allelomorph, the fact that the male side of gynandromorphs did not have the maternal, recessive autosomal character effectively disposed of Boveri's hypothesis of partial fertilization. On the other hand, when a recessive autosomal gene entered from the father's side and its dominant allelomorph from the mother's side, the fact that the male side of the gynandromorphs did not show the paternal, recessive character likewise eliminated Morgan's earlier hypothesis of polyspermic fertilization. It has been shown, further, from crosses, the parents of which differed in sex-linked characters, that maternal and paternal X chromosomes are eliminated with about equal frequency.

In certain experiments with *Drosophila,* in which a determination of the frequency of sex-chromosome elimination was undertaken, it was found that one gynandromorph appeared in about every 2,200 individuals. Since only those individuals that start as females give the kind of gynandromorphs observed in these tests, it was concluded that one case of chromosome elimination occurs in about 1,100 individuals.

Of the evidence from plant material there is the recent account by Frost (1921) of the occurrence of a bud sport in *Matthiola* in which presumably linked genes have segregated out simultaneously in one or more branches. While this case will require further investigation before the manner of its origin can be positively established, it seems probable that it belongs to the category of somatic segregation by chromosome elimination or non-disjunction.

Studies of mosaic endosperm of maize afford perhaps the most definite evidence available in plants that certain somatic variations are due to aberrant chromosome behavior such as non-disjunction or elimination (Emerson, 1921). The genetic evidence that I have been able to obtain in support of this interpretation is of much the same

nature as that noted above for *Drosophila* gynandromorphs. In crosses in which recessive aleurone and endosperm characters are contributed by the female parent and their dominant allelomorphs by the male parent, spots of the recessive (maternal) aleurone color are underlaid by the recessive (maternal) type of endosperm when the genes for these aleurone and endosperm characters are genetically linked, that is, when they are carried in the same chromosome. On the contrary, similar recessive (maternal) aleurone-color spots are always underlaid by the dominant (paternal) type of endosperm when the genes are not linked, that is, when they are carried in non-homologous chromosomes. The fact that linked genes separate out simultaneously while non-linked ones do not do so supports the view that mosaic seeds are the result of some chromosome aberration such as elimination or non-disjunction, and renders untenable the earlier hypotheses of incomplete fusion of endosperm nuclei suggested by Correns and by Webber and also that of gene mutation proposed by myself.

The work with aberrant maize endosperm has furnished an opportunity to study the frequency of chromosome aberrations in a specialized tissue. The available data show that when a single chromosome alone is concerned, about one mosaic seed occurs in every 420 seeds. If the other two homologous chromosomes of any one set are involved as frequently and if any one of the ten triploid chromosome sets is as likely to be involved as any other one, one case of aberrant chromosome behavior should occur in about every fourteen seeds. There is some evidence, though not convincing as yet, that in different strains of maize chromosome aberrations may occur with strikingly different frequencies. In one culture in which only a single chromosome could have been involved in the origin of mosaic seeds, as many as twenty-five such seeds have been observed on a single ear of approximately 500 seeds, or one for each 20 seeds. If this behavior proves to be a constant one in this strain and if the other 29 chromosomes behave in like manner, it should furnish excellent material for cytological investigation. Moreover, the possibility of the existence of

strains of maize differing so widely in the frequency of chromosome elimination or non-disjunction raises interesting questions concerning the causes of such aberrations. It would seem possible to determine by appropriate tests something as to the relative influence of maternal and of paternal contributions on the rate of chromosome elimination.

There are circumstances connected with these results from *Drosophila* and *Zea* that may raise some doubt of their general applicability to cases of bud variation. The *Drosophila* evidence is limited almost exclusively to the sex chromosomes, though there is no positive evidence that elimination may not occur among autosomes and result in non-viable individuals. The data from *Zea* relates to endosperm alone, a specialized, nutritive, sterile, triploid tissue. There is perhaps justification for a belief that the sex chromosomes of animals and the triploid chromosomes of the endosperm of angiosperms may be subject to irregularities in behavior not commonly found in other material. The only answer to such a contention is (1) that gynandromorphs and endosperm mosaics are the materials that have been critically studied and (2) that there is, or should be, no presumption in favor of vegetative segregation through chromosome elimination or through other means as against vegetative mutation or any other mechanism as a possible explanation of bud variations that have not been subjected to cytological investigation or to critical genetic analysis.

Cytoplasmic Segregation.—Numerous cases of apparent segregation of cytoplasmic elements have been reported in plants. Of these, examples from *Mirabilis, Pelargonium, Primula,* and *Zea* may be noted. All of them involve visible effects on chlorophyll development and all show non-Mendelian inheritance.

Correns (1909*a, b*) working with a white-spotted-leaved type of *Mirabilis* observed a very irregular distribution of the white and green areas, each varying from small spots to whole branches. These white and green characters were found to be inherited through the mother only. The situation with respect to *Pelargonium,* re-

ported by Baur (1909), differs from that in *Mirabilis* in that the spotting is transmitted through the pollen as well as through the egg cells. Spotting appeared in F_1 in crosses of white with green without respect to which way the cross was made. As in *Mirabilis,* wholly white and wholly green, as well as mosaic, branches were observed.

Examples of maternally inherited chlorophyll variegation have been investigated by Gregory (1915) in *Primula,* and by Anderson[3] in *Zea.* The genetic behavior of these materials is quite the same as that of Correns's *Mirabilis* variegation. The apparent difference in the cytological basis of their behavior, however, must not be overlooked.

Evidently these plants of *Mirabilis, Pelargonium, Primula,* and *Zea* are sectorial chimæras. Their main interest in connection with this discussion lies in the fact that, starting with a single fertilized egg cell, certain chlorophyll deficiencies are apparently separated out into certain vegetative cells and handed on through definite cell lines, while normal chlorophyll develops in other cell lines, with the result that areas of varying extent have one or the other of these characters. In what the mechanism of this segregation consists—if segregation it be—is not in all cases certainly known. It may even be that some cases of variegated chlorophyll are to be regarded as recurrent variations arising *de novo* after the manner of somatic mutations but effecting changes in the cytoplasm, or some of its inclusions, rather than in the chromosomes. Baur is inclined to the view that in mosaic plants of *Pelargonium* deformed chloroplasts are responsible for the chlorophyll deficiencies and that these are segregated out by chance in cell division. This view is supported by Gregory, who noted in the young leaves of variegated plants of *Primula* the existence of normal and chlorotic plastids in the same cells. Correns does not commit himself to any particular element or inclusion of cytoplasm as the seat of the cause of chlorophyll deficiency. Randolph (1922), from cytological examination of Anderson's striped leaved maize, found that, in the

[3] Unpublished data.

transition regions between the green and the pale-green areas, the cells contain not only green and colorless plastids, but all intermediate conditions as well. Since the green and the white plastids are not two sharply differentiated kinds, but are the end members of a continuous series arising from minute primordia which, so far as can be seen, are of one kind, he regards any simple form of segregation hypothesis as inadequate. It seems possible, however, that these primordia may be functionally, even though not morphologically, of two more or less distinct classes.

Graft-hybrids and Other Chimæras.—The well-known graft-hybrids of *Solanum* reported by Winkler are of interest from the standpoint of this discussion because of the bud variations commonly exhibited by them. Sectorial chimæras, produced by adventitious buds arising from the point of union of stock and scion of grafts of tomato and nightshade, and having one side of the one species and the other side of the other, have not infrequently later produced branches that were periclinal chimæras having tissues of one species enclosed within an envelope of the other. That these branches are really periclinal chimæras has been established by chromosome counts and by the fact that seedlings produced by them are always of the species of the subepidermal tissue from which gametes arise. These periclinal chimæras in turn have been observed to produce branches wholly of one or other of the parent species. The marked difference in appearance between the sectorial and periclinal chimæras and between the latter and either parent species places this behavior clearly in the class of bud variation and, since the production of branches of the parent species from periclinal chimæras is the result of a separation of genotypes that were closely united previously, the phenomenon is perhaps rightly classed as a form of vegetative segregation. It is obvious, however, that the separation of tissues that are merely closely associated in the graft hybrid is a fundamentally different type of segregation from that by which the chromosomes or even the

plastids or other cytoplasmic elements of a single cell are dissociated.

The behavior of "natural" periclinal chimæras of *Pelargonium,* noted by Baur (1909), and of *Pelargonium* and several other forms, described by Bateson (1919), all of which involve green and white regions of the plants and some of which produce reverse periclinal chimæras, is fundamentally the same as that of graft-hybrids. The manner of origin of these natural chimæras is unknown, but it is quite possible that they arose as somatic mutations.

The case of *Bouvardia* also, as reported by Bateson (1916), is presumably of quite the same order as the examples noted above, though its behavior is strikingly different in detail. Varieties of *Bouvardia* that are maintained true to type by propagation from stem cuttings produce plants with very different flower form, size, and color when propagated by root cuttings. While this behavior is not to be taken as positive proof that these varieties are natural periclinal chimæras, it is quite in keeping with such an assumption. Since in normally produced buds of the stem both the epidermis and the deeper lying tissues are maintained through direct cell lineage, while the roots produced by stem cuttings arise from the plerome and break through the periblem and dermatogen, forming these parts anew, sprouts that develop from the roots must have the genotype of the stele rather than that of the cortex or epidermis.

From the results of critical investigations cited in this account, it is evident that vegetative variations are due to diverse causes. Some are certainly due to somatic mutation of genes; others are as certainly due to chromosome aberrations; and still others have been somewhat definitely shown to involve a vegetative segregation of plastids or other cytoplasmic elements. There are many problems relating to these several types of behavior that are in great need of further critical study both genetic and cytological. The results of future research will depend in large measure on the choice of favorable material. Quantitative data are of the greatest importance

and from this standpoint no material gives more promise of fruitful results than that involving variegation.

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SEROLOGICAL REACTIONS AS A PROBABLE CAUSE OF VARIATIONS

PROFESSOR M. F. GUYER

UNIVERSITY OF WISCONSIN

WITH an insight that has never been surpassed even to this day Claude Bernard,[1] more than forty years ago, remarked that "Organic synthesis, generation, regeneration, maintenance, and healing of wounds, are different aspects of an identical phenomenon," the phenomenon alluded to being the constructive activity manifested in ordinary nutritive processes. In a recent thoughtful paper, R. S. Lillie[2] reiterates and expands this point of view. That synthetic metabolism constitutes the very essence of embryonic development and therefore of the expression of heredity, scarcely admits of a doubt. To-day it is a truism to say that the visible "characters" we deal with in heredity are but the effects—by-products as it were—of far-reaching metabolic reactions. And since the metabolism of the actual living protoplasm centers, if not exclusively, at least principally, in the proteins, the problems of metabolism, growth, reproduction and heredity become largely the problem of why and how a given kind of living protoplasm builds up proteins of its own specific type.

The molecules of the ordinary native proteins are, as is well known, huge polymeric structures of extremely complex constitution. By appropriate chemical treatment they may be broken down into successively smaller and smaller units, each of which, however, still responds to the ordinary qualitative tests for proteins. There finally comes a point below which further reduction of the molecule results in the loss of the distinctive protein reaction, and the outcome is a series of ultimate characteristic units, the amino-acids. Thus native proteins seem to be built up of two different categories of units: first, combinations of various amino-acids which constitute the simplest protein blocks; and second, the combination of these into the much larger molecules characteristic of the native proteins.

1 "Leçons sur les phénomènes de la vie," Vol. II, p. 517.

2 *Biol. Bull.*, XXXIV, 2, 1918.

In the process of digestion different proteins are broken down into their amino-acid units and these are then rebuilt into the tissue-proteins of the living organism, each tissue selecting such amino-acids as are required to reconstruct its own peculiar complex. That is, the architecture of the new proteins into which the individual building units are regrouped is determined by the specific constitution of the tissue-proteins themselves. In different proteins the different amino-acids may exist in very different ratios, and certain of them necessary for the metabolic repair of protoplasm may be lacking in some, such as gelatin, but the amino-acids in any particular protein are constant in nature and proportion and each probably has a definite position in the molecule. While kinds and proportions of amino-acid units determine in large measure the characteristics of individual proteins, it may well be that configurational differences in molecules of the same chemical composition are responsible for the specificities of corresponding proteins in related species of animals. One estimate, for instance, assigns to the serum-albumin molecule alone the capability of having as many as ten thousand million stereoisomers. One may perhaps picture mentally, in a much simplified form, the simplest protein molecule as a main chain or ring, of which the representative links are amino-acid "nuclei." Moreover, to each such link

(*e.g.*, in simplest form, $\text{—NH.}\underset{\underset{\text{H}}{|}}{\text{CH}}\text{.CO—}$)

a side-chain, differing in constitution in different cases, is attached or is attachable by replacement of a hydrogen atom.

It is, then, with such complex molecular configurations that we have to do as a chemical basis for the phenomena of life; and in them, as I have stated elsewhere,[3] we have "ample basis for that peculiar handing on of metabolic energies already established which we term *heredity*."

Although habitually when speaking of heredity we think of the multifarious "characters" displayed by the adult organism as the things inherited, and strive to picture in our minds how they are represented in the germ, it is

[3] Amer. Nat., XLV, May, 1911.

clear, in the light of modern genetics, embryology and cytology, that what actually happens is a reduplication generation after generation of germinal protoplasm. That is, the proteins already present in the germ-cell not only determine what will be built up in growth, but also the composition of that overgrowth which, as a detached individual, constitutes the physical basis of inheritance. If the initial protoplasmic substance is chemically specific then inevitably the anatomical and physiological complexities which arise out of it must likewise be specific.

Before we can grapple with the problem of the possible induction of changes in this fundamental mechanism through influences emanating from the body, we must consider some matters concerned with embryonic development and the fundamental chemical nature of the somatic cells.

As to how the constitution of the egg becomes transformed into that of the adult, the most consistent and reasonable hypothesis to date, in my opinion, is that proposed by Child, based on axial or metabolic gradients. A full exposition of his hypothesis must be sought in his books, "Individuality in Organisms" and "The Origin and Development of the Nervous System from a Physiological Viewpoint." I can sketch only such aspects of it as pertain to my present subject. Starting with the universally accepted biological axiom that excitability followed by some degree of transmissibility is a fundamental property of all living matter, Child believes, as I understand him, that the establishment of polarity in the fundamental organismic proteins of the germ-cell is the beginning of development. The eggs of many species already show polarity (animal and vegetal pole) at the time of ovulation; in other forms polarity is not established until later. In the former case the polarity may have been determined in earlier cell-generations by extrinsic factors or it may be due to the original position of the ovum in the ovary with reference to the nutritive stream. Yolk apparently accumulates in the region of least oxidation and thus marks the vegetal pole. In the second type of egg, polarity, at first lacking, is soon established because differential environmental exposure (difference in oxygen supply, light, contact, general surface exposure,

or other external factor or factors) causes one region to have the greatest metabolic activity. As does any stimulated part with reference to a resting part (muscle, nerve, etc.), this point of heightened activity sets the pace, as it were, for the other parts. Thus an excitation gradient is established which Child terms an *axial gradient.* Since the excitation initiates transmission—changes and thereby determines what shall happen at successive levels along the path of transmission, the region of highest excitation dominating and controlling the rest, the axis in question may also be called a *metabolic gradient.* In this way a physiological unity is established and maintained by bringing the different regions within range of the gradient into definite physiological relations.

Since the chief source of energy in protoplasm is oxidation, and inasmuch as many different tests have shown that the rate of oxidation gradually diminishes along the gradient from the region of highest activity, Child infers that differences in oxygen supply play a very important part in the local metabolic differences which arise. In any event, a differential axis of activity arises in the egg as the result of differences in environmental conditions (either before or after ovulation) and determines the axiate pattern of the developing organism. In such a complex system of chemical and physical activities, where unquestionably many associated simultaneous reactions and interactions are going on, different rates or conditions of reaction in different regions must result in unlike end-products. Thus, at different levels of a gradient which was quantitative in origin, *qualitative* differences arise. For once a gradient is established, any one of several purely quantitative changes in the system, such as increased oxidation which acts differentially on substances at a given point, changes in temperature, in water content, or in colloidal state, or changes in the concentration of the reacting substances, may alter certain component factors of a given region more than it does the corresponding factors in other regions, with the result that out of the same initial constituents the respective organ- or tissue-stuffs that characterize the organism are gradually built up. For example, as Child points out,

in a region of rapid oxidation certain substances might be entirely oxidized as rapidly as they are formed, while in a region of slower oxidation they might accumulate as part of the structure.

Bilateral organisms follow a law of antero-posterior development. Differential exposure having determined which region shall lead and having thus set the rate of activity of the successive regions, continuing differential relations of the environment maintain the various levels of the gradient, under the domination of the head-end, at their respective rates of activity. While this is the normal course of development, it may be greatly altered by experimental methods; the original gradient, particularly in lower organisms, may be obliterated and a new one engendered. The latter under certain conditions may even be made to arise at right angles to the original axis. Change of gradient may be readily observed, for example, in the regulatory development of isolated pieces of many planarians. Moreover, the remarkable capacity for self-differentiation possessed by isolated parts taken at different levels of the body in such forms, shows that positional relations of the constituents of the regenerating mass, rather than cellular specificity, determine what structures shall arise in a given location.

Since many species, including representatives from all the chief phyla of animals, show differential susceptibility at some stage of their development, when subjected to the action of various external agents such as alcohol, anæsthetics or potassium cyanide, these agents may be used to bring about shifts of gradient, under-development or over-development of certain parts, or a remodelling of the organism or of various regions of it. Inasmuch as any one of several agents may bring about the same result, it is manifest, again, that quantitative external conditions are the factors which initiate and thereby determine the fundamental orientations and specializations of the parts of an organism.

As development progresses in the more complex organisms, again through differential stimulation, secondary or "symmetry" gradients may also become established. For example, each limb region of a vertebrate becomes a subordinate system with its own internal correlations.

In organisms with radial symmetry, special centers of growth occur, and only at a certain distance from a given center can another arise.

After differences in protoplasmic constitution have arisen at different levels of the gradient, a system of chemical or transportative correlation probably begins to operate, and out of it all finally comes the various supportive and mechanical tissues, vascular tissues, tissues of excretion and secretion, nervous tissues, etc., which constitute the underlying mechanisms of correlation and integration in the finished organism.

The hypothesis says little of chromosomes, or of genes, for its objective differs somewhat from that of the geneticist, but it in no wise denies the existence of such entities. Child argues, however, that since each cell of the body is a descendant of the original zygote and therefore presumably possesses the full complement of chromosomes of that zygote, something other than the nuclear pattern *per se* must be responsible for the fact that cells become different in different parts of the body. And this something, he would say, is the metabolic gradient initiated by differential excitation, since the very establishment of such a gradient means the concomitant establishment of local differences along its path. As he says, "if all the cells are originally alike they cannot of themselves become different." The specific character of the differentiation, the kind of organ or organism produced, he reiterates, is determined "by the specific inherited constitution of the protoplasm."

So much for Child's hypothesis of axial or metabolic gradients, in its bearings on embryogeny and differentiation. I have reviewed it at some length because it shows more clearly than any other theory of development with which I am acquainted that there is no necessity for believing that as cells become specialized they lose part of their original constituents. Due to local conditions, structural modifications and special activities have appeared, but these are changes rung on what is fundamentally the same type of protoplasm in every cell. In higher organisms, in some cells possibly irreversible changes have occurred—the cell may be incapable of dedifferentiation —but nothing constitutional, call it gene or what you will,

has necessarily been lost from the cell. The inheritance complex of the germ is like goods in the piece; it is only as development progresses that the garment becomes specified; but above all, be it remembered that the finished garment is of the same fundamental constitution as the goods in the piece.

It is a commonplace of experimental embryology and experimental morphology, in fact, that the same initial materials may yield very different end-products in different environments. The phenomena of heteromorphosis, metaplasia, regeneration and regulation all attest this. Blastomeres originally directed toward becoming one part of an organism may be switched about to become another part; tissues originally subserving one function may be turned to other uses; ectodermal cells which by no possible chance could have been predestined to form crystalline lens will, nevertheless, form a lens similar to that of the normal eye if stimulated by a transplanted optic cup. Or, if the lens is removed from the eye of a salamander, a new lens may develop from the edge of the old iris, a part from which the lens never normally develops in the embryo. In short, it is a well-established fact in many organisms that cells occupied with the specializations of one part of the individual still retain the potentialities which would fit them to the functions of some different part, and may, in fact, under experimental conditions be made to redifferentiate into the structures of another part. Such facts, together with the exactitude of chromosome distribution in mitosis, indicate clearly that many, possibly all, cells of an organism retain the hereditary tendencies that existed in the original zygote. Because of limitations due to its location in the organism, however, a given cell realizes only a small proportion of its inherent possibilities. And after all, this is no more remarkable than the fact that the genes of recessive characters may slumber indefinitely in germ and soma, generation after generation, until conditions suitable for their expression as characters occur.

But now, regarding heredity as in its simplest expression merely the passing on of metabolic activities already established, and conceding that the distinctive structural

effects and functions which characterize the respective tissues are probably the outcome of unequal activities among the same kinds of fundamental protoplasmic constituents in differing local environments, the question of prime importance to the student of evolution is how the properties of these constituents have come to be changed from what they were initially, how they may be altered in the future—in short, the question of the nature and origin of variations. For whatever we may believe about the degree of preformation which exists to-day in the mechanism of heredity, it is absurd to assume that in the simpler primitive protoplasm from which modern forms have evolved there could have been genes of the characteristics of all the organisms now in existence. Whatever individual development may be, we must assume that racial evolution was epigenetic. While doubtless in a sense man lived potentially in some primitive protozoan-like creature, actual material antecedents of his existing attributes were no more present in this ancestral creature than specific determiners for the oceans, continents and topographical features of the world to-day were present in the original nebula which preceded our solar system. The great central problem of evolution is just this very one of how the determinative accumulations which exist in germ-cells to-day have been incorporated step by step into this erstwhile primitive protoplasm. Certain possibilities have become realities and concomitantly as a basis of this reality the old mechanism has in part been altered, or a new mechanism has come into being which persists as a part of the established constitution of the germ-cell.

Before entering upon a discussion of whether or not any of the remarkable serological activities which have come to light in recent years may be possible or probable sources of germinal modifications, we must recall briefly the general nature of immunologic reactions. As you know, foreign proteins of either plant or animal origin when injected directly or indirectly into the circulation of an animal will engender antagonistic or neutralizing substances to which the general name of *antibodies* is applied. Thus the toxins of bacteria incite the production of *antitoxins;* the bacteria themselves lead to the pro-

duction of bacterial immobilizers or solvents termed *bacteriolysins,* or sometimes to agglutinating substances termed *agglutinins* which clump bacteria of the species used in their production, if the two are brought together in the blood-serum of the animal into which the bacteria were originally introduced. Likewise a tissue of one kind of animal injected into the circulation of another induces the formation of antibodies of various kinds such as *precipitins* which form a precipitate when the blood-serum of the treated animal and an extract of the special tissue used are brought together in vitro; or other antibodies termed *cytotoxins* or *cytolysins* which possess a specific toxic or solvent action for the kind of protein used in their production. The alien substance employed to produce antibodies is commonly called the *antigen.*

In this connection, the phenomenon of anaphylaxis should perhaps also be mentioned. Anaphylaxis is a name given by Richet to designate a highly supersensitive state which, after a period of incubation, an animal develops toward certain protein substances that were practically harmless on first injection. Sometimes, particularly in guinea pigs, death results. The sensitizing dose for the production of anaphylaxis may be very small; one millionth cubic centimeter of horse-serum, for example, has been known to render guinea pigs sensitive. The reaction is specific; for instance, an animal sensitized to sheep-serum, though reacting violently to this antigen, displays little or no hypersusceptibility to other sera.

In the main all of the immunological reactions show a considerable degree of specificity; the antibody will react fully only with the particular kind of protein used as antigen. The specificity is not absolute, however; a milder reaction may be obtained with homologous proteins of related species, the extent of the reaction being determined by the nearness of relationship of the species to that from which the original antigen was obtained. Similarly with bacteria, the reaction is in the main specific, although so-called group-reactions may appear. The serum of an animal immunized against typhoid, for example, may not only agglutinate *Bacillus typhosus* but may also show this reaction in a less degree with such related forms as the colon bacillus. Thus, ir-

respective of whether the antigen consists of bacteria or of other protein materials, there is a gradational specificity of reactions which apparently corresponds to taxonomic relationships.

An even more delicate biochemical measure of kinship than the known immunological reactions has apparently been established through the extensive researches of Leo Loeb[4] and his associates on transplanted tissues. In numerous of his papers Loeb has called attention to the remarkable power of transplanted tissues to indicate different degrees of even close individual relationship, such as the individual to itself, to a brother or sister, to a parent, to a more distantly related individual of the same species, or to an individual of a different species. Particularly the lymphocytes of the host serve as a delicate indicator of such relationships.

Loeb assumes that a specific chemical group which he designates as *individuality-differential* is common to all the tissues of an individual and that in virtue of this characteristic each creature differs from the others of the same species. The individuality-differential of a transplant (except in autotransplantation), since it is not adapted to its new environment, assumes injurious properties, probably by engendering toxins. The relative strengths of these are determined by the degree of relationship that exists between the source of the transplant and the host. In the circulating fluids of a given individual, he believes that there are "autosubstances" which exercise important regulative functions, such, for example, as keeping the vascular supply of the various tissues at an optimum, or holding in check lymphocytes and invasive fibroblasts which when inadequately restrained, as in old age, become destructive agents. In one place he speaks of their stimulating effects and he regards them as responsible directly or indirectly for the marked vascular reaction called forth by autotransplants.

In sexual reproduction, obviously two different "individuality-differentials" must combine to form the new individuality-differentials of the offspring. These, Loeb finds, are of varying degrees of intermediacy. This intermediacy is continued into the next generation. What

[4] AMER. NAT., LIV, 1920.

is of much interest from the standpoint of our quest of a possible connection between reaction-products of the body and alterations of the germ is the fact that he feels constrained to link up his serumal phenomena with the chromosomes. Thus, he says, "The chemical individuality-character of the chromosomes should lead to analogous chemical differences consisting perhaps in the formation of chemical side-chains attached to proteins; they should be present primarily in cell-proteins and secondarily in the proteins of the body-fluids. . . . These side-chains must be identical in all the proteins of the same individual and differ in the case of different individuals."

Another great group of influences which extend to the furthermost reaches of the body and profoundly affect the entire organism in development and in maturity—those emanating from the various endocrine structures—I have barely time to mention. They must be kept in mind, however, when we attempt to picture the ebb and flow of chemical influence which is indispensable to the maintenance of general physiological equilibrium, including that of the gonads no less than of the other body structures.

You may feel that in reviewing the nature of the protein molecule, the behavior of the proteins of the cells in morphogenesis, the gradational specificities of the immunological reactions, the relationships which exist between host and transplant, and in reminding you of the intricate functions of the endocrines, I have wandered far afield into irrelevant byways, but I hasten to assert that these phenomena are not as unrelated as might appear at first sight; they are but different aspects of the great salient fact of organismic unity, whether it be a matter of chemical constitution, taxonomic relationship or physiologic response.

And now I wish to raise the question of whether or not in the light of the foregoing facts it is irrational to believe that in all probability a thread of chemical identity persists between the chemical constituents of the germ and the chemical substratum of the tissue-cells. The nuclei of the various tissue-cells differ little in appearance from the nuclei of the germ-cells, and inasmuch as the new germinal and somatic cells descend alike

directly from a common source, presumably bearing in their chromosomes samples of all the chromosomal components of the original zygote, is it unreasonable to suppose that if changes come to pass which can affect certain constituents of tissue-cells, this influence, if borne in the circulating fluids of the body, could also affect the homologous constituents of the germ-cells? Personally, I think that such a hypothesis is not unreasonable. But is there even the least bit of evidence on this point? I believe that there is. I feel that in the transmission of eye-defects secured by Dr. E. A. Smith and myself in fetal rabbits by means of serum immunized against rabbit crystalline lens, we have a bona fide case of such parallel influences. Since I have already presented the facts before this Society and inasmuch as the details are available in printed form,[5] I need not repeat them now. It is sufficient to recall to you that we secured a fowl-serum immunized against rabbit crystalline lens which when injected into pregnant rabbits penetrated the placenta and occasionally attacked the lens of the fetal young, the outcome being marked eye-anomalies in such young. Since, once produced, the defects were transmitted to successive generations through both male and female lines, we interpreted our results to mean that the immune serum was not only specifically cytolytic for the newly forming lens-tissue of the fetus, but that it also attacked the representatives of such tissue—its genes, if you please—in at least some of the germ-cells of the fetus. If true, this must mean that there is some degree of constitutional identity, probably protein homology, between the mature substance of a tissue and its correlative in the germ. And in view of the fact that, basically, inheritance is mainly a question of the perpetuation of specific protein-complexes, and development, the result of differential reactions of these same fundamental constituents under differing conditions of environment, is this an unreasonable inference?

But does anything comparable to this occur in the ordinary course of animal existence? Do cytolysins or kindred substances which can modify or destroy both

[5] *Jour. Exp. Zool.*, 31, 2, 1920. AMER. NAT., LV, 1921. *Proc. Nat. Acad. Sci.*, 6, 3, 1920.

tissue-elements and their germinal correlatives ever occur in animals without being introduced by man? Do animals ever form such antibodies or other equally active substances against their own tissues? It is obvious, since tissues persist intact under conditions of normal physiological equilibrium, that they are not being subjected to such influences, or if they are, that they resist them. As a matter of fact, Römer,[6] using the complement-fixation technique, found that the serum of adult human beings may possess antibodies for their own lens proteins. It seems reasonable to suppose that if the tissues of an animal became injured or displaced in some way, or metabolically unbalanced, immunity reactions might be established against them. We have some evidence that such is the case. During the late war, for example, it was found that toxic reactions resembling anaphylactic shock often followed extensive injuries of the soft tissues. The matter can be tested experimentally. Because of their distinctive nature and the ease with which they may be isolated, I chose spermatozoa for such an experiment.[7] I found that a rabbit will build antibodies against its own spermatozoa when these are injected into its blood-stream; also, that rabbits injected with rabbit spermatozoa not only develop antibodies in their blood, but also have their own spermatozoa greatly weakened, a condition shown in vitro by their lessened resistance to antisera. This clearly shows that an animal can on occasion build antibodies against its own tissues; and since antibodies can apparently directly or indirectly affect germ-cells, it seems reasonable to suppose that such influences, especially if continued over a long period of time, might be one source of germinal variations.

It is known from the experiments of Kuntz[8] and others that the blocking off of the ductus deferens of one testis may induce degeneration of the germinal epithelium, not only of that testis, but of the other as well. Inasmuch as the spermatozoa in the testis on the operated side must die and be resorbed, is it not probable that in this process spermatotoxins have been formed which have then attacked the living germ-cells of the other testis? Again, we are familiar with the fact that oculists fre-

[6] Zinsser: "Infection and Resistance."

[7] Paper in press, *Jour. Exp. Zool.*

[8] *Anat. Rec.*, 17, 4, 1919.

quently find it necessary to remove a severely injured eye to prevent the "sympathetic" degeneration of the other eye. I am told by competent oculists that the extension of the degenerative influence involves more than the atrophic effects which might result through direct nerve connections. Does it not seem probable that here, too, the disintegrative influence which comes to operate on the uninjured eye is cytotoxic or cytolytic in nature? And if it can operate on the tissues of the normal eye, why not on the corresponding protein constituents in the germ, the prototypes of those which were originally incited to form the ocular tissues?

It may be, it probably is true that there is sufficient difference between these factors of the germinal protoplasm and those of the finished organ to render the former less susceptible to such agents. It is not improbable that even if some of the numerous germ-cells were affected many others might not be. But any new organism which sprang from such an affected germ would have its own germ-cells similarly modified, since these would all be derived from the same zygote. Even so, the defects might not be manifested in offspring because of the probability of dominance by the corresponding factors from their partner in fertilization.

The only way to settle the matter, of course, is through experiment. I know of no existing experimental evidence on this point. In my own laboratory, however, an investigator has an experiment in progress which I hope will ultimately throw some light on the matter.

There are many bits of evidence to show that an organism may react against the tissues of other individuals of its own species. Thus Bradley and Sansum,[9] employing anaphylactic reactions, found that guinea pigs injected with various guinea-pig tissues such as heart, liver, muscle, testicle, and kidney developed immunity reactions. Moreover, certain changes in the blood of the mother during pregnancy, apparently induced by cells or cell-products set free from the newly-forming placenta, seem to be of the nature of antibody formation. Then again Turck[10] has shown that products of the lung-tissue of the cat, autolysed under sterile conditions in *vitro*, pro-

[9] *Jour. Biol. Chem.*, Vol. 18, 1914.
[10] *Med. Rec.*, 1919, 95, pp. 719–21.

duced characteristic pulmonary lesions when injected into other cats. Similarly autolysed lung-tissue of other mammals had no effect on cats.

But, the question arises, in order to get parallel influences, in soma and germ, would there not have to be absolute identity between the two sets of proteins concerned? Before answering this question let us glance for a minute at two types of specificity which are recognized in serological reactions: namely, "species-specificity" and "organ-specificity." What the serologist means by species-specificity is the fact, shown through precipitin reactions, that blood immunized against one tissue of an alien species will react, although in a less degree, with extracts of the other tissues of that species. And that there may be a specificity of certain organ complexes which is independent of species is shown by the fact that an immune serum produced by using the crystalline lens of one species of animal yields a precipitin which reacts more or less with the lens proteins of even unrelated species. Similar results have been obtained with proteins derived from the testis, and confirmatory evidence of such organ-specificity has also been established by means of anaphylactic reactions. Such facts as these, together with those cited in the discussion of the gradational reactions of various immune sera according to the systematic relationships of animals, it seems to me, answer our question affirmatively; there need not be absolute identity between the proteins of the somatic cells and their correlatives in the germ-cells for immune sera engendered against the one to react also against the other. I raise the issue because it might be urged that such tissues of an organism as become so abnormal as to excite the production of antibodies are no longer sufficiently similar to the normal tissue-elements, and therefore to their germinal representatives, to make the antibodies effective against either normal somatic or germinal constituents.

It seems to me that all available facts indicate that the constitution of an organism, whether germ or soma, is not to be regarded as a congeries of cooperating, equipotent units, but rather as the outcome of interacting systems which differ in their orders of organization; systems which in themselves possess more fundamental and

more supplementary or fluctuating components; chemical groups which represent the more constant features of organization coupled with subsidiary groups of more restricted significance. That is, there seem to be series of substances of like chemical constitution common to all the cells of an organism, possibly to even various groups of organisms, and superimposed upon these central or foundational constituents, probably as parts of the same molecules, are secondary systems, or possibly systems within systems, which modify the main configurations in various ways.

This conception certainly squares with the fact that degrees of specificity paralleling the kinships of animals may be shown by immune sera. It harmonizes with what we know of the architecture of the native proteins as well as with our whole scheme of natural biological taxonomy in which we find certain fundamental stable features representing a broad series of organisms, and less and less inclusive characteristics which grade down to the minor differences that separate species, varieties and individuals. Nor is it incompatible with what we know of chromosomes and genes. The very fact that heritable grades of a single gene in a given chromosome may occur (*e.g.*, in *Drosophila*) and that one of these variants may in turn be modified gradationally by a series of secondary factors located in other chromosomes suggests the type of organization just discussed.

With the remarkable and abundant evidence of hand-and-glove relationships between unit-characters and chromosomes that has been accumulated in recent years through the painstaking studies of workers on *Drosophila*, not to mention other corroborative work, it seems to me that there is no longer a reasonable doubt that the differentials, whatever they may be, responsible for the distinctiveness of the so-called unit-characters, reside in the chromosomes. And while I have always believed[11] and still believe that for the final outcome the cytoplasm is just as necessary in its way, and must be just as characteristic of the species as the chromosomes are, its distinctiveness must be of a fundamental organismic (probably chemical) type common to the species as a whole,

[11] Bull. No. 2, Univ. of Cincinnati, Vol. III, Ser. II, 1902. *Science*, June 28, 1907. Univ. Cincinnati Studies, Sept.–Oct., 1909. AMER. NAT., XLV, 1911.

since it can be, in fact is, contributed in reproduction almost wholly by one parent, the female. It is apparently a medium which responds specifically to the action of the respective chromosomal incitants, whether these be of maternal or paternal origin. All geneticists agree to-day, I think, that any character of an adult can not be merely the outcome of a unitary germinal antecedent; it is the product of many factors. And ordinarily what we see as a character-difference is probably merely the outcome of a factor-difference in one of the chromosomal cooperants.

In conclusion, let me say first of all that no one more than myself realizes the inadequacy of my present argument as a complete or satisfying theory. The knowledge in the fields on which it is based is as yet far too fragmentary to warrant anything but tentative conclusions. But since various facts seem to me to point toward the view that certain types of immunological reactions, notably the cytolytic, engendered against various somatic constituents may occasionally also affect chemically related substances in the germ, and inasmuch as many other facts lend themselves to such an interpretation without undue violence to scientific credulity, I have felt justified in presenting the whole matter in the form of a working hypothesis.

In the short time remaining I can not enter into the important question of whether or not changes induced in the blood-serum might be instrumental in leading to progressive rather than regressive evolution, and even had I time for such a discussion, there are not sufficient data available to support such a discussion affirmatively. I should like merely to point out in closing that through exercise we can initiate and promote growth in various parts of the soma, we can induce hypertrophy, and in so doing we are in some way leading the protein and other constituents of the cells in question to make more of their own kind of substance, in other words, to reproduce their kind. We do not know what stimulates them to do so, but, in part, it may well be something that is or can be transported in the circulating fluids of the body; and if so, then there exists the possibility that the corresponding germinal representative of such a part, however tenuous the thread of chemical connection, might also be modified in the direction of progressive germinal change.

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ORTHOGENESIS

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ORTHOGENESIS FROM THE STANDPOINT OF THE BIOCHEMIST

PROFESSOR L. J. HENDERSON

Harvard University

It does not seem likely that physical science should have much to say about the theory of orthogenesis. In the first place, it is hard to see what the term means if one adopts a physico-chemical standpoint. In the second place, organic evolution is more remarkable in its morphological aspects than in its chemical and physico-chemical aspects.

I

The first point may be dismissed with a few remarks. Orthogenesis presumably means that evolution has taken place in a straight line or in a very restricted path, and that the straightness of the line depends, at least partly, upon something which is internal to the organism, though, of course, the process may be released by a stimulus from the environment. The straightness of the process must be largely a matter of definition. Physico-chemically, it could hardly mean more than that quantitative changes have steadily the same sign over a considerable period of time.

One might, perhaps, adopt such a view, if one could believe, as has been often suggested, that variation is the expression of a process which is approaching a condition

of equilibrium, because then, so far as there is no untoward interference from without, it would be natural to think that the course of the process must be in a certain sense a straight one, with a negative acceleration. Taken literally, such a consideration is, however, purely speculative and for the present, I think, a sterile speculation.

Somewhat more clearly intelligible is a hypothesis which arises from the study of hormones and their rôle in development. It appears to be quite possible that the effect of increase or decrease in the amount of a single chemical substance in a species might be a complex change in its structure, including modifications of size, of the proportions of the different parts, of pigmentation, or of the other peculiarities which ordinarily arrest the attention of students of evolution. This would be especially true if, instead of a change in the amount of a hormone or other substance, it were a case of the formation of a new compound. Such changes, while directly due to a single substance, might be greatly modified by readjustments following the disturbances of the physiological equilibria between the different parts of the body. Compensatory readjustments of similar nature are, of course, among the most familiar and interesting phenomena in pathology. We are, accordingly, fully justified in taking their possibility for granted.

It is, therefore, conceivable that evolutionary changes should be occasionally progressive and apparently orthogenetic, although due to a simple physico-chemical modification. No doubt, if it were desirable, such considerations might be developed into a clear and possibly useful theory of orthogenesis, but I am not qualified to do so. My object is only to insist that changes which from a morphological standpoint are complex, continuous, and progressive, may conceivably be due to a single, simple, physico-chemical change.

Such reflections, vague though they may be, clearly point to a conclusion which is, I feel sure, inevitable for the physical scientist; morphological phenomena in them-

selves are not sufficient to establish the validity of any theory of the mechanism of evolutionary variation.

II

More important than speculating about such questions is the fact that the underlying physico-chemical processes in living organisms seem to have remained about the same throughout the whole process of evolution. So far as it is possible to form any opinion on the matter, this conclusion is inevitable.

In considering the question of organic evolution it should always be remembered that, with very trivial exceptions, the economy of life on the earth is now and probably always has been founded upon the synthesis of carbohydrates from water and carbonic acid with the accompanying fixation of energy, followed by the conversion of carbohydrates into fats, proteins, and a great variety of other related substances. Later there is an oxidation of these substances back to water and carbon dioxide, accompanied by the utilization of the energy in various forms of organic activity. Correlated with this is the fact that cells are made up of water, carbonic acid, carbohydrates, fats, proteins, and certain other substances. They are enough alike in chemical composition and in physico-chemical structure fully to justify the concept of protoplasm as a fairly constant physico-chemical apparatus throughout the organic world.

These familiar facts of chemical physiology and chemical morphology undoubtedly depend upon the properties of the substances involved. Water and carbonic acid, with which the process begins and ends, and which seem to be everywhere the foundation of protoplasm, possess in themselves such a large number of remarkable characteristics and lead directly through the formation of sugars to such a great variety of chemical substances and chemical reactions, that it is hard to believe in the possibility of the existence on a large scale of any very different kinds of living organisms.

This is a subject that I have elsewhere discussed at length. Hence it will perhaps suffice briefly to recapitulate a few of the more striking facts. Because of its peculiarities as a solvent, as an ionizing medium, etc., water makes possible the formation of an almost indefinitely greater variety of physico-chemical systems than does any other substance. On account of its high latent heat of vaporization, its high specific heat, its high surface tension, and the peculiarities of carbonic acid, such systems often possess a very remarkable stability. The elements hydrogen, carbon, and oxygen, of which water and carbon dioxide are composed, seem to be unique in the number and variety of the substances which they can form. In particular, the production of sugar from water and carbon dioxide fixes a very great amount of energy and leads directly to the greatest variety of chemical substances and reactions which are known to occur as the result of one chemical process. Finally, water and carbon dioxide are the two substances which are everywhere available.

Anything so complex, so stable and yet so variable, so widespread and so active as life can only occur when a great variety of conditions are fulfilled. In other words, the physico-chemical systems of the organism, in order that life shall be capable of its evolution, must possess altogether exceptional characteristics, which appear to be quite impossible unless water and carbonic acid, and compounds of the three elements, hydrogen, carbon, and oxygen, and no doubt also of nitrogen, are at the basis of them. These substances possess a set of properties each one of which by itself and also in cooperation with the others is necessary for the highest physico-chemical complexity and variability. So far as we know, no other elements or compounds possess another set of properties which permit similar physico-chemical complexity or variability.

It is, I believe, for this reason that life has always operated on the same basis.

Thus while the evolutionary process has certainly produced a large number of well-defined series of changes when it is looked at from the morphological point of view, it still remains very probable that such physico-chemical changes as have occurred are not only of a secondary nature, but that they are much less of the character of serial modifications. Indeed, one is tempted to say that in a physico-chemical sense, the variations are distributed in rather a random manner, without any particular indication of a general progressive tendency, such as we seem obliged to think of in studying morphological variation.

No doubt the evolutionary process has, from time to time, invented new chemical substances and greatly modified colloidal systems. In the total these changes are very numerous and of the utmost importance to the student of evolution. But *progressive* change is more particularly a morphological phenomenon and it seems to be almost self-evident that progressive morphological evolution should not be accompanied by the same degree of continuous variation in straight lines in physico-chemical properties. Such a parallelism would, I think, be well nigh unaccountable. However that may be, there is no evidence for it.

III

Another consideration which makes the theory of orthogenesis seem very different to a physical chemist from what it must seem to a biologist, is the fact that chemistry tends to deal with individual substances which either exist or do not exist. The case of hemoglobin will illustrate this point. Hemoglobin is an individual substance of very marked peculiarities. So far as known there are no essential differences between the hemoglobins contained in the bloods of different species. It is possible that the known differences in crystal form depend upon something more than trivial differences in the amino acids which make up the molecule, but this seems unlikely. In any case, it will do no harm to speak of hemoglobin as

a single chemical individual in order to illustrate a particular point.

This substance is the sole means of transporting more than a small amount of dissolved oxygen in the blood of those species which contain it. It is, therefore, apparent that it may be thought about from the evolutionary point of view, much as one thinks about an organ. I believe that the success of Aristotle's system of classification justifies this view. But while it is easy to think of the gradual evolution of an organ as something which can not be regarded as appearing at any point in the evolutionary process, being related by a process of continuous differentiation to something which was certainly not the same organ in an ancestral species, there is not the slightest evidence for anything of the sort in the case of hemoglobin, and it must seem to most chemists nothing less than fantastic to assume such a continuous evolution of a substance more and more closely approaching hemoglobin. Moreover, it is almost as difficult to imagine such a thing from the standpoint of a biologist, and it is certainly true that any given organism either does or does not contain a substance which is capable of forming a loose chemical combination with oxygen.

But the difficulty in the case of hemoglobin is more serious than this, for it has been found that hemoglobin, like other organs, has more than one function. It has, in fact, at least three; for it is the sole means of transporting oxygen, almost the sole means of liberating carbonic acid in the lung and absorbing it in the tissues, and the instrument of the final delicate adjustment of the alkalinity of the blood. The last two functions depend upon the same property in the hemoglobin molecule, but this property is a different one from that which enables hemoglobin to combine with oxygen. We are, therefore, here confronted with the task of imagining the origin of a chemical substance, quite different in its nature from any other known substance, which possesses two chemical peculiarities, and which, as a result of these two peculiarities, performs three highly important functions.

Now it may be that originally hemoglobin possessed only one of these peculiarities, so that its sole original function was to carry oxygen. And accordingly one of the most interesting questions of comparative physiological chemistry concerns the respiratory function of the blood. It would be a very important discovery to find a kind of hemoglobin in which there is no specialized action upon the transport of carbonic acid and upon the alkalinity of the blood. But even if the earliest hemoglobin were of such a nature, the first production of hemoglobin would still seem to have been relatively an extremely discontinuous variation involving an unmistakable physiological unit of great importance.

It is true, and should be noted in order to avoid confusion, that there has been a later evolution of the process of oxygen transport. This has been commented on by Barcroft as a result of his own important researches. It appears that variation in the electrolytes of the red cells is accompanied by remarkable variation in the affinity of their hemoglobin for oxygen, and that this is the explanation of the differences in the so-called oxygen dissociation curves of the bloods of different species of mammals. Here, as Barcroft points out, there is no difficulty in imagining a process of adaptation, for the fact of chemical discontinuity is not involved. It is a question of changing proportions of the different substances.

But in spite of the possibility of such phenomena, it seems probable that there are, even in the human species alone, a considerable number of important individual substances whose appearance in the course of organic evolution it is very difficult to imagine, except as a radical innovation.

Accordingly, it must be apparent that in the present state of our knowledge, any theory which postulates continuity in evolution is very unsatisfactory to the chemist.

Moreover, in this case one seems to be confronted with an appearnace of discontinuity which does not depend, as is too often the case, upon a judgment of the magnitude of a difference.

Of course, it is not difficult to imagine a sufficiently close approach to continuity of evolution, and therefore, to orthogenesis, in the case of simple proteins. But here, very likely on account of our ignorance, there is no indication of anything more than indefinite variation and variability, accompanying variation in a definite direction in the morphological characteristics of species.

On the whole, variation in the ultimate physico-chemical nature of organisms seems to have been rather discrete than continuous, not orthogenetic, but distributed at random. Such a conclusion may possibly be illusory, for our ignorance is greater than our knowledge. But whatever the nature of the changes which it has undergone, the most striking thing about the physico-chemical nature of protoplasm seems to be its uniformity throughout nature.

Therefore, with due reservations because of the incompleteness of bio-chemical knowledge, it seems reasonable to suppose that apparent instances of orthogenesis may sometimes depend upon a single important chemical change in an organism, followed by slow and progressive modifications leading up to a definitive morphological result. Such a process would be somewhat analogous to the establishment of a condition of equilibrium.

ORTHOGENESIS IN BACTERIA

PROFESSOR CHAS. B. LIPMAN

UNIVERSITY OF CALIFORNIA

It is well to understand at the outset that bacteria, unlike plants and animals, can not be studied from the orthogenetic standpoint in the strict sense, owing to the lack of a proper vantage point, or, perhaps more correctly speaking, a basis from which one may start such a study. In my opinion, all attemps at the establishment of systems of bacteria, and there have been many, have ended in creating greater confusion than there was at the start. Such frustration of well-intentioned programs was inevitable for at least three reasons, to wit: (1) Every bacteriologist used criteria of his own for the establishment of new species. This is bound to lead to chaos. (2) Nearly all bacteriologists used a morphological basis for classification. Owing to the relative simplicity of form of bacteria, this must inevitably result in erroneous discriminations. (3) Nearly all bacteriologists in the past and even to-day are laboring under the misconception that bacteria are simpler forms of living organisms than they really are. That this is incorrect has been shown by the studies of Löhnis and Smith in 1916[1] and of Löhnis alone very recently.[2]

But assuming the foregoing to be true, it must follow that it is impossible to trace the evolution of new species of bacteria in a definitely directed course. Not being certain what constitutes a forward or what a backward step

[1] (*a*) "Life Cycles of the Bacteria," preliminary communication, *Jour. Agric. Res.*, Vol. 6, No. 18. (*b*) "Life Cycles of the Bacteria," paper read at New Haven meeting of Soc. Amer. Bacteriologists, Dec. 27, 1916.

[2] "Studies upon the Life Cycles of the Bacteria," Part I, Review of the Literature, 1838–1918, *Nat. Acad. Sciences*, Vol. XVI, Second Memoir.

in bacterial development, it is obvious that we can not apply as justifiably as we do in the case of the higher organisms the criteria of definitely directed evolution. To be sure, we have a number of instances of the establishment of permanent new characters in bacteria, yeasts, and other fungi through the influence of a change in environment. Most of these are, however, induced through changes in the medium under artificial conditions and they do not necessarily indicate a change in the direction of improvement of the organism or of greater complexity in its organization which may in turn point to the evolution of a higher form from a lower one.

Despite the foregoing, it is probably well to examine into certain facts with which we are familiar with regard to microorganisms, and which may, perhaps, have a close bearing upon what might be regarded as orthogenesis in bacteria. The first fact to which I wish to refer is that of parasitism. There can probably be very little doubt that parasitism on the part of bacterial cells is not an original, but an acquired character, using the term "acquired" in its literal, and not technical, sense. If that is granted, it would also follow that the acquirement of such a characteristic by a microorganism would mean the gradual adaptation of a bacterial cell from one kind of a medium to another. It would mean the gradual acquirement of partiality on the part of a microorganism towards certain chemical substances, certain temperatures, or certain other conditions which obtain only in a living host and not in an inanimate medium. The steps, gradual or rapid, by which the acquirement of such peculiar characteristics on the part of the microorganism would occur in its change from a saprophyte to a parasite would almost seem to imply evolution in a definite direction. In a sense, therefore, we may regard parasitism in bacteria as an evidence of orthogenetic development in such organism. It is, moreover, a case of evolution in a definite direction through the influences of environmental factors of the natural order and not those

which are produced artificially. In respect, therefore, of causal factors in the evolution of bacteria, we have parasitism exemplifying the antithesis, so to speak, of changes which we induce in bacteria in our artificial media, or by changes in the environment. These observations would seem to possess cogency, not only in the case of obligate parasitism, such as that characterizing the organism of human tuberculosis, or of anthrax, or of the fungus of wheat rust, but also that of what we may call facultative parasitism in which the organism may have adapted itself to life, both as a saprophyte and as a parasite through the influence of certain chemical or physical-chemical agencies in its environment which have rendered its protoplasm more highly adaptable than that of the obligate parasite. We may, therefore, regard facultative parasitism as an instance of orthogenetic evolution, just as we may so regard obligate parasitism. The puzzling question which may, however, arise, from these considerations is, which is the more advanced step in orthogenesis in parasitic bacteria. Is the obligate parasite the more advanced form, or is the facultative parasite the more advanced form? While many would probably, on first impulse, regard the former as the correct answer, it does not necessarily follow that such is the case. Certainly in this regard, a great many more facts are needed before any definite statements can be made.

Examples of other cases of orthogenetic evolution in bacteria other than the case of parasitism, which I have just discussed, may be multiplied *ad libitum*. But, owing to limitations of time and space, it will suffice to mention a few only.

The adaptation of bacteria to the physiological characteristic of nitrogen fixation, such as is possessed by all the *Azotobacter* species, and the *Clostridium* species and, to a slight extent, by many other species, can scarcely have been the result of anything else than a case of definitely directed evolution. This was probably accomplished through the influence of an environment in which

it was impossible for the organisms existing therein to live without acquiring a power of employing energy existent in carbonaceous material to fix atmospheric nitrogen and make it available for their own life processes. The next case which may be cited is that of the lactic-acid bacteria, which possess the power of transforming lactose (or milk sugar) into lactic acid. These cells are not in form or otherwise in function appreciably different from any other bacteria with which we are acquainted. They have, nevertheless, this specific and peculiar power to which I have alluded. Is it likely that they have acquired this power through any other influence than the influence of environment which operated in a definite direction and hence orthogenetically? The sulphur bacteria, or particularly those species of sulphur bacteria which have the power of oxidizing sulphur to sulphuric acid, are another case in point. The nitrifying bacteria are still another case in point. The iron oxidizing bacteria are still another case, and so we might go on and mention very many classes of bacteria, in each case of which there is a definite, distinct, and strikingly peculiar functional power which could not well have been developed without the influence of some environmental factor or factors operating in a definite direction. It is not so easy on the morphological side to give examples like those which I have just cited from the point of view of function of the bacteria. The reason for that has already been touched on above, namely, the simplicity of form and particularly the slight variety in form which characterizes the bacteria. In fact, it is my conviction that it is best to ignore, largely, morphological factors in bacteria when we study the problem of bacterial evolution. My conviction arises from a study of many and varied experiments which I can not discuss here.

Viewing our subject, then, from the standpoint that orthogenesis in bacteria would be concerned with progressive changes in the organism, principally physiological, due to its response to changes in environment, it

seems that we must admit that orthogenesis does exist there. But if, on the other hand, progressive changes like those in question must also be in the direction of producing a more advanced form of organism, we are confronted by a quandary resulting from a lack of an accepted definition for the term "advanced."

The argument that bacteria do not *at all* lend themselves to appraisal as regards evolution by the standards applying to the higher organisms is, perhaps, not sound as shown again by the researches of Löhnis, which I have just cited. The objection to viewing bacteria in a manner similar to the higher organisms because no sexual reproduction is known among them is removed by Löhnis' observations, which indicate that something akin to a real conjugation of cells does occur in the bacteria. His striking monograph in the Memoirs of the National Academy should be read and studied by all those who seek new light on the origin and nature of bacteria.

Another point of view which I believe may be introduced into this discussion with some justification results from a broad comparison of natural phenomena generally. In the inanimate world, we are confronted by the evolution of substances in series in which the first member of the series is simple and by small accretions becomes progressively more complex in the succeeding members of the series until very complex materials are finally built up. We are all well acquainted with the seriation showing progressive complexity in the hydrocarbons beginning with methane and going up; in the carbohydrates beginning with formaldehyde and going up; in the proteins beginning perhaps with amino acids and going up. Such examples of progressive seriation may be multiplied *ad libitum*. Why, therefore, should it not be possible that similar series should arise in the progressive evolution of bacteria through certain forces as yet largely unknown which cause accretions of characters, so to speak, to occur in bacteria through their being rendered more complex and complicated by the influence of

certain factors of the environment. It seems inconceivable to me that the great diversity and complexity of functional nature in the bacteria could have arisen otherwise. Nevertheless, analogies between phenomena in animate and inanimate nature must not be pushed too far in the absence of the necessary facts for their support. While I believe them to be of great significance, I do not desire to be dogmatic on the subject in the slightest degree.

While all the foregoing as regards the evidence for orthogenesis may be accepted as true, it does not follow that the doctrine of orthogenesis is anything new or significant or was so when it was first enunciated. It seems to me to constitute merely one way of describing the actual condition of progressive series in evolution, but it seems to me that it *explains* nothing. In so far, however, as its advocates espouse the cause of those who believe in and give evidence for the inheritance of acquired characteristics, the potency of environment in inducing fundamental and permanent changes in the organism, and the theory of mutation, they do contribute something significant to the discussions and experiments which constitute the amorphous symplasm, metaphorically speaking, from which our knowledge of the well-defined and real nature and origin of life may some day be expected to emerge.

It is, perhaps, of particular importance now to consider the bacteria as a class and their probable origin as bearing on the question for which we are trying to find an answer. There is a general disposition, and particularly is it true that there has been in the past, on the part of biologists and natural philosophers, so called, to place the bacteria in point of origin among the most primitive of living organisms. There is much inclination, indeed, to regard them as *the* most primitive organisms. While, superficially, this view seems attractive and correct, it loses much of its cogency when one takes into consideration the following situation: In all but a few

exceptional forms of bacteria, some of which I have named above, the physiological characteristic is either that of a saprophyte or of a parasite. It seems obvious to me that neither a saprophytic nor a parasitic organism can well be expected to originate in an environment which is devoid of elaborated organic matter. Subject to considerations which I shall discuss later, we must, therefore, accept one or two conclusions with regard to the origin of bacteria in the scale of evolution of organisms generally. Either they are the most primitive forms of organisms which have lost their primitive powers of living in purely inorganic media, or they are a much more advanced form of life which came to be after other organic forms had for some time been developing on the earth's surface. The first possibility is merely tantamount to saying that some cells of the most primitive forms have gradually adapted themselves to either a saprophytic or a parasitic existence and, therefore, is of little assistance to us. The correctness of the second conclusion, however, would seem to depend on many little-known factors. Still, it is the belief of many scholars. Putting the matter in another way for greater clarity and emphasis, I may state it as the opinion of several plant physiologists who have speculated upon this subject, that the primitive forms of living cells were probably those which could live in a purely inorganic medium. Obviously, such cells must have been limited to the group which we now call the autotrophic organisms, and of the autotrophic organisms, since the higher plants are certainly a very advanced form, we must have had something very much simpler, and the natural conclusion is that such a simpler form of organism must have been the single-celled green alga, or forms closely similar to it. If we assume that such was the case, then it is not difficult to propose a scheme of evolution of the bacteria which involves the gradual change of the unicellular green algæ into a variety of bacterial forms through the influences of environmental factors as I have already in-

dicated. It is not at all inconceivable that a green algal cell may have adapted itself gradually to life within a higher plant cell or within an animal cell, or to a saprophytic existence in soil or other media devoid of light. It may first have come there accidentally and then, through the power to respond to such an environment and to tolerate it, has gradually evolved new powers and has lost some of its old powers. It is conceivable, therefore, that whether we regard parasites and saprophytes among the bacteria as degraded forms or not, they may be examples of evolution in a definite direction, presumably in this case in the direction of greater complexity of function resulting from the urge of a constantly and markedly changing and potent environment.

Since the foregoing observations on orthogenesis in bacteria have led me to enunciate in another form a theory accounting for the origin of bacterial forms which has been discussed before, I feel constrained to go one step farther into that subject in order that my own views may not be misunderstood. While the idea of accounting for the origin of the bacterial cell from the single-cell alga seems attractive and appears to be in consonance with certain well-known facts, there are several troublesome features about it. In the first place, it assumes the development of so complicated and intricate a substance as chlorophyll before any form of living substance was evolved. While this may, of course, have been the case, it seems doubtful, in view of what we must consider to be the highly specialized nature of the green pigment of plants. In the second place, we have seen that the strong argument in favor of the theory of the single-celled alga as the primordial cell, or rather against the theory that bacteria may have been such primordial cells, lies in the well-known fact that most bacteria require organic compounds as energy for their life processes and that no organic matter could have been available without the activity of chlorophyllous organisms. This argument, however, overlooks two points, viz., first, the existence of

autotrophic bacteria and, second, the possibility and even probability that sufficient amounts of organic matter for bacterial purposes may have been elaborated at the dawn of life by chemical means, using the term "chemical" in the broadest sense. It is, of course, well known that the autotrophic bacteria, for example, the nitrifying bacteria, can live and build organic matter out of purely inorganic substances, carbon being obtained from carbon dioxide of the air, and in the absence of light and chlorophyll. But if this is so, why may it not be that of the known forms of living cells, the autotrophic bacteria were the first, since they are capable of living in a purely inorganic medium, the ammonia which is necessary to them being supplied from the small amounts resulting from chemical reactions induced by electrical phenomena in the atmosphere. As we have seen thus far, it may be argued, with equal justice, that the activity of the nitrifying bacteria is a highly specialized one on the one hand, and a very primitive one on the other.

But if, as just indicated, it should be argued that, after all, the autotrophic bacteria are exceptions in the bacterial world and that most bacteria need elaborated organic matter and hence they could not have been the primordial living cells, the second objection which I have stated may be urged, namely, that organic matter may have existed on the earth before living cells came into being. Mature reflection will render it highly plausible with the high temperatures, great electrical activity, and probable intense radioactivity which existed on the planet prior to the appearance of living cells, that unusual chemical activity inducing rapid and general combinations among the elements should have prevailed. This, moreover, involves the assumption of the existence of a degree of all these conditions which is requisite for the synthesis, but not for the rapid destruction of the organic matter, which must also be conceded as probable. Under such conditions, it is reasonable to suppose that bacteria, on being evolved as the primordial cells, may

have found the conditions requisite to their growth and further development.

It seems, on careful deliberation, that strong arguments may be brought forward for both the theory that single-celled green algæ and the theory that bacteria were the primordial organisms, if we consider merely the arguments which enter into the usual discussions of the subject. But it appears to me that we must penetrate beyond what is ordinarily called careful deliberation, if we would see other possibilities for explaining the origin of living matter. There is no logical reason for confining our attention in these discussions to the single-celled algæ and the bacteria which we know. There are, in addition, bacteria so small as to challenge and defy our ingenuity for devising means for rendering them visible. What may not further discoveries about their nature and requirements for life unearth for us which may be of the most vital significance to the solution of our problem? I have tried in imagination to go beyond, far beyond, the ultramicroscopic bacteria and have pictured to myself the following condition for the origin of living matter: A single molecule of organic matter, let us say, a polypeptid or a proteid molecule produced by the force which I have discussed, exerted as chemical energy, may, in floating about in its aqueous medium on the earth's surface, suddenly find itself in a field of radioactive force or some similar force which causes its atoms to orient themselves in such fashion and to vibrate in such a manner as to endow it with certain activities which we now regard as attributes of life. Crude though this conception may be, it constitutes a step, though perhaps a very bold one, into the realm of possibilities for explaining the origin of the first living cell, a subject which we must consider together with all our theories of evolution if we do not wish to remove the inspiration to progress by arriving at an impasse in our theories and our hypotheses.

In conclusion, it is well to review briefly the discussion which I have just presented in a very brief form. Out

of regard for your time and patience, I have merely presented in outline each of the important considerations which I deem of direct significance to the question at issue. I have presented the difficulties which lie in the path of treating bacteria from the point of view of orthogenesis, and yet have shown that they may be so treated with certain justifiable assumptions as a basis. Having thus treated them, however, I have shown that whether the theory of orthogenesis holds for bacteria or not, it can not be considered as explaining anything, but merely as a mode of describing our observations. I have gone into the more fascinating and what seems to me to be the more useful discussion of the origins of living cells and the position of the bacteria with regard to such primordial cells. I have mentioned the various hypotheses which, in my opinion, may be considered to be the most plausible in that connection, and have shown the weaknesses and the strength of each. It has been my purpose to give an unbiased presentation of my own hypotheses and those of others without prejudice to any so that you might be enabled to discuss them all and arrive at your own conclusions. Without a thorough review of the literature of bacterial physiology and morphology, it is not easy to obtain a broad enough view of the subject to do it justice and I would urge particularly that those who are interested in it acquaint themselves with the absorbing and inspiring literature of the subject of mutation in microorganisms. I believe that it is full of significance for biological progress and I wish that circumstances made it possible for me to present a brief review of it for your consideration. As it is, I must content myself with directing your attention to it and with expressing the hope that my humble efforts in preparing and presenting this paper will constitute a step forward in our progress of thought and experimentation on problems in the evolution of living matter.

ORTHOGENESIS AND SEROLOGICAL PHENOMENA

PROFESSOR M. F. GUYER

University of Wisconsin

As my discussion progresses I fear that some of my hearers may be reminded of the old joke about the mongoose. A stranger carrying an odd-looking box was asked by a man whose curiosity got the better of his good manners, what was in the box. The stranger replied that it was a mongoose and went on to explain that his brother was subject to delirium tremens, during the attacks of which he believed that he was being strangled by snakes; this mongoose was to catch the snakes. To the reminder by the inquisitive man that these were imaginary snakes he retorted, " Yes, I know, but this is an imaginary mongoose."

Since some of our most competent investigators in the fields of genetics and evolution are skeptical apparently about the whole question of orthogenesis, to them, at least, I shall be making an imaginary attack upon a mythical phenomenon. President Kofoid, however, seemed to think that some of the recent work with immune sera done in my laboratory, which strongly indicates the induction of permanent germinal modifications, might have possible theoretical implications bearing on the question of orthogenesis, and I agreed to discuss the subject, although realizing at the outset that the net result would not be a scientific proof, but merely a suggestion which might possibly be of some value as one of various working hypotheses.

First as to orthogenesis itself; is there such a process? Our answer must depend largely upon how we define orthogenesis. It takes but a glance at the literature of the subject to see that it has meant many different things to many different people, ranging from a mystical inner

perfecting principle, to merely a general trend in development due to the natural constitutional restrictions of the germinal materials, or to the physical limitations imposed by a narrow environment. In most modern statements of the theory, the idea of continuous and progressive change in one or more characters, due according to some to internal factors, according to others to external causes—evolution in a "straight line"—seems to be the central idea. To many, faced by the seeming impossibility of explaining by natural selection the origins of new characters, it has been apparently merely a welcome general utility concept by which one may account for the beginnings of new organs, or the development of parts along definite lines, irrespective of utility.

For present purposes nothing is to be gained by a review of the different theories of orthogenesis, all so well summarized in Kellogg's "Darwinism To-day," and I shall proceed merely on the assumption that, judging from the statistical law of errors, certain variations are apparently not fortuitous, since they tend to accumulate in certain directions. It is customary to add that the lines of development which result are independent of, and in extreme cases may be opposed to, the operation of natural selection. I see no reason, however, for believing that if variations occur in definite directions of no use to the organism, why they may not also occur just as definitely in directions which lend themselves to the perfecting influences of natural selection. The difficulty in determining this point lies in the fact that an evolution based on the selection of even fortuitous variations must in one sense be orthogenetic, that is, along definite lines, so that there is no way in retrospect of telling whether the underlying germinal variations were purely fortuitous, or whether they were biased toward an adaptive outcome.

Of the various lines of evidence brought forward in support of theories of orthogenesis, the ones which appeal most convincingly to me are: (1) those based on

parallelisms in variation which appear in different branches of the same large group of organisms; (2) those argued from the premise that the very nature of the chemical complexes which constitute the body of a living organism necessarily limit changes to relatively few directions; and (3) some of those instanced in the field of palæontology.

As to examples of parallelisms in related forms, I am most familiar with conditions to be seen in the color patterns of the pheasants (*Phasianinæ*) and the guineas (*Numidinæ*). In a paper[1] written some years ago I summarized my observation on various features of the coloration in a number of groups of genera and species in these two subfamilies as follows:

> . . . there are certain basic tendencies for particular elements of the coloration, such as the formation of eye-spots, barring, and the like, to follow along definite paths of development. When arranged with reference to one of these elements, such, for example, as barring, which is one of the most universal, instead of possessing distinct and unrelated markings, the different species in a given group are seen to be standing merely at different levels in the development of one, or at most a few, continuous progressions of the special pattern in question. Since when so grouped the gradation in pattern is as much in evidence between collateral kinsmen as between those of direct lineage, one can only conclude that the bias toward a particular line of patterns is the product of fundamental protoplasmic peculiarities implanted in the group as a whole.

Further on in the same paper it is shown that where the pattern has become obscured it may be brought to expression again through hybridization. In the interesting group known as the peacock pheasants (*Polyplectron*) which by systematists is regarded as intermediate between the peafowls and the pheasants in the narrower sense, the varying stages and types of ocellation to be seen afford a good illustration of the point at issue. Quoting again from this earlier study:

> Again as regards ocelli or "eye-spots" in *P. chalcurus*, which appears to be the most generalized species, one finds no ocellation. The only hint of what is to be realized in the more specialized

[1] *Jour. Exp. Zool.*, VII, 4, 1909.

members of the group is found in a pronounced purplish and greenish "metallic" coloration present on certain feathers of the tail. In the male of *P. emphanes*, while there are numerous green metallic iridescent areas on the feathers of the upper wings and back, they have not yet progressed to the condition of being definite ocelli, although on the tail of this same individual there are two transverse bands (the one on the retrices, the other on the upper tail coverts) of ocelli. Still a step in advance, in the male of *P. thibetanum*, Gm. (*P. alboocellatum* Cuv.; type, Mus. d'hist. nat., Paris) the small feathers of the wings and the feathers of the inter-scapular region bear distinct small purple ocelli ringed successively with black, light brown, and white. The tail is also banded with ocelli. In the male of *P. germaini* the wing-coverts and back bear numerous green ocelli. The female of this species, as usual less advanced phylogenetically than the male, has the ocelli of the body much less distinctly marked.

An idea frequently attached to the theory of orthogenesis, yet which I believe is in no wise a necessary part of it, is that the various grades of a feature supposed to show orthogenesis have arisen as a connected succession, the supposedly most advanced stage having emerged from the stage next in order below it. That the individuals of a large group which show some particular characteristic expressed in different degree can be arranged in a gradational series with reference to that characteristic is obvious, but, as pointed out by various critics of the theory, this does not prove that the various expressions of the character in question arose thus sequentially. In a recent study yet unpublished, made by Miss Sarah V. Jones in the department of genetics at the University of Wisconsin, on the genetical behavior of checks and bars in inheritance in pigeons, for example, Miss Jones corroborates the results obtained by Staples-Browne and by Bonhote and Smalley which show that the two patterns are independently inherited in Mendelian fashion with checked-wing dominant to barred. She also found the relation of uniform black to check to be one of simple dominance, and furthermore, that certain grades of checking are inherited independently. But she found no evidence in support of the well-known view of Whitman that

the various grades of checks form a series moving in one direction, the ultimate outcome of which is the two-barred and finally barless types. And Miss Jones points out that it does not "necessarily follow that because the interaction of these several factors produces an apparent epistatic series, the mutations producing the various grades of checking should have occurred in any particular order."

And here, to my mind, is the crux of the matter. In order to have what may legitimately be termed orthogenesis, do the underlying mutations have to occur in any particular order? Is not the very fact that, instead of existing as a medley of wholly unrelated elements, certain characteristics of organisms, such as color markings, can frequently be arranged as parts of a definite pattern or as stages in a general process—does not this in itself indicate directional variation? When one sees in its incipiency, as it were, in one species a character which has attained to an advanced expression in a kindred group, especially where there are intermediate expressions of the same characteristic in other related species, is not this indicative of a general trend in variation? For instance, is not the tendency toward the formation of "eye-spots" in the plumage of the pheasants hinted at even in the greenish-black iridescence so often visible in the tail feathers of the common rooster—is not this tendency the expression of what in last analysis must be a germinal bias? To be sure, this bias finds different ranges of expression in different species: as eye-spots on the wing-feathers in some species, on the body-feathers in others. In one species they may occur as a single row of ocelli, in another as two rows, across the tail. And it is obvious that certain of these patterns have not been derived directly from others, since they appear in what are clearly collateral lines. Nevertheless, the tendency to form ocellations is present in many if not all species of this great group. We know nothing of the order in which the mutations occurred which brought about any partic-

ular condition that at present exists in the group. Some of them may have been small and sequentially related, and it is not impossible that the extremes were thrown in one line, while grades of less advanced type came into expression in collateral lines. It is also clear that even should certain grades have arisen as a progressive series there is no reason, from the viewpoint of the mutation theory, why any two particular grades should not show the characteristics of Mendelian unit-characters, irrespective of the order of their origin. The important point is that in this group when mutations occur in certain color pattern-controlling factors, whether great or small, they tend toward the formation of eye-spots in some degree.

While we know little of the chemistry of animal pigments, the reactions involved in color-production in certain plants are better understood, since many of the pigments have been extracted and analyzed. Along with the understanding of structure that has been gained in the chemical studies of synthetic dye-stuffs, has come considerable knowledge of the relation between color and molecular structure. In many cases, for instance, as Nietzki[2] has shown, where the pigments of most simple construction are yellow, by increase of molecular weight they change to red, next to violet, then to blue. A good review of the theories of color in organic compounds, given as an introduction to her own painstaking and valuable chemical researches[2] on "Pigments of Flowering Plants," will be found in a recent paper by Dr. Nellie A. Wakeman. Most of the information about organic pigments used in the present discussion has been obtained from this source.

Upon reading such a piece of investigation together with the accompanying discussion of related studies one is impressed by the fact that a comparatively few processes underlie most pigmentary changes in plants. Enzymes—hydrolases, reductases and oxidases—frequently

[2] *Trans. Wis. Acad.*, XIX, Part II, pp. 767–906, Madison, Wis.

play an important part in the formation of pigments, or in changes in pigments already formed. Shade of color, for example, is evidently often merely a function of oxidase content. With a graded increase of oxidase, therefore, a plant might be put through a regular gamut of color effects. In general, the mere addition of hydrogen to dye-stuffs reduces them to the corresponding leuco base. Armstrong, in his quinone theory of color, makes much of the quinones as the colored compounds in dye-stuffs and maintains that the corresponding colorless compounds are hydro-quinones. The structure and size of the pigment molecule itself seems to be an important factor in color. Hydrocarbon radicals, for instance, deepen the tint; the addition of hydrogen raises the tint. In analyzing any particular case one has to take into account the original molecule, the position of any group introduced and the number of groups introduced. It is possible, for example, by the introduction of a tint-deepening group to deepen the color, but by introducing two or three more such groups to throw the absorption wholly outside the visible spectrum and thus do away with the color. From this it is clear that two compounds may be closely related in constitution and yet one be colored, the other colorless. As to why, physically, in both the aromatic and the aliphatic series, color is produced in certain compounds and not in others, various investigators have arrived at the conclusion that the cause of color is due to "the making and breaking of contact between atoms, thus giving them marked activity," a process known as *isorropesis*, and Miss Wakeman goes on to explain:

> This change of linkage which must accompany the transformation of one modification of the compound to the other is the source of the oscillations producing the absorption bands. If these oscillations are synchronous with light waves of a high frequency they give rise to absorption bands in the ultra violet and the compound is colorless. If, however, they are less frequent, the absorption band appears in the visible portion of the spectrum and this absorption of colored rays results in the compound taking on complementary color.

Among interesting facts that come to light in Miss Wakeman's summarization may be mentioned the following: all organic pigment molecules are unsaturated; the quinone grouping is one of the best known of the chromophorous groups; by far the largest number of plant pigments are referable to hydrocarbons of saturation C_nH_{2n-14} and C_nH_{2n-16}; and finally, that it is the relation of the chromophorous groups to each other and to the rest of the molecule, and not their mere presence in the molecule, that postulates color in a substance.

I may seem to have dwelt unduly upon pigmentation in plants but I have tried to go into the matter only sufficiently to give a glimpse of some of the real conditions which underlie some of the "unit-characters" we are juggling about in genetics, and around which we are attempting to frame hypotheses of evolution. Color, perhaps more than any other one thing, has in recent years been utilized for genetical observations. And when it is known that in many cases color is merely a function of the degree of oxidation of some fundamental compound, or the introduction or subtraction of some hydrocarbon radical, it does not tax the imagination to conceive of how it is possible to have series of color "characters" that in the parlance of organic evolution represent orthogenetic series. As simple a matter as the relative degree of oxygen supply, probably determined by the amount of an oxidase present, may account for various stages of color. It is manifest, moreover, that in a given group there might easily be a tendency toward increase in oxidase-production, which if present unequally in different collateral lines might give us just the uneven condition, with different species standing at different levels of expression of the trait in question, which exists in various alleged cases of orthogenesis. It is obvious, furthermore, that any higher state of development of the character in a particular species need not have sprung from the next lower stage, but may have had its immediate origin from any level of the scale.

Most of the constitutional changes which go on in the living organism seem to center in the proteins of the protoplasm. Metabolism is largely a question of the disruption and reconstruction of the various cell-proteins. In the cell, moreover, the characteristic protein-complexes themselves determine the nature of the synthesis that shall go on. When synthetic activity is more than sufficient to make good metabolic waste, growth results, and when such increase becomes overgrowth and takes the form of a detached individual, we pronounce it reproduction. We are then in position to talk about inheritance—the fact that a new individual possesses the properties and, under similar conditions, therefore, will express the activities and take on the appearances of the earlier or parent form. Thus the germ-cell is a reduplication of the zygote from which it sprang, a detached bit of living matter made up largely of certain characteristic protein-complexes. Even the simplest protein is a huge molecule built up of a series of different kinds of amino-acid " nuclei " which in different proteins differ in numbers, kinds and arrangements. Certain of them seem necessary to all proteins, others are present in only some proteins. Each amino-acid, besides being linked to its fellow, has a replaceable hydrogen atom which may be exchanged for any one of several radicals or " side-chains." Furthermore, the ordinary native proteins are secondarily compounded of a number of the simpler blocks formed of linked amino-acids. The unitary amino-acids which enter the blood as protein digestion-products are used as building units again, each cell selecting the kind of units it requires for replacements in its own proteins.

We have no reason to believe that the proteins of the germ-cells have any mysterious powers associated with them that are not shared by any or all of the somatic cells. The modern view of embryogenesis and histogenesis no longer finds it necessary to picture troops of pangenes departing from their home in the nucleus at just the proper time to take possession of the cytoplasm

and turn the cell into a specific tissue-cell. Each tissue-cell probably retains all the essential properties of the original fertilized ovum from which it has directly descended. In many cases, among lower organisms, at least, we know that somatic cells detached as buds or experimentally can through regulation and new growth reconstruct themselves into complete organisms. That a particular cell takes on the characteristics of a specific tissue seems to be determined by the special environment in which the cells happen to be placed in the organism.[3]

Exactly how much chemical difference there is between two unlike tissues or between the cells of a particular tissue and the germ-cells is not known. As far as we have any cytological evidence to the contrary the nuclei, at least, of the tissue-cells are not essentially different from the nuclei of the germ-cells. While the various tissues differ very much in appearance, this is mainly the result of the accumulations of intercellular products or of cytoplasmic modifications. And many of the latter may be largely changes in colloidal state rather than fundamental changes in chemical composition. In any event, the new condition is one which has sprung from a cellular chemical constitution similar to that of the original zygote. And if this is true, would not any internal or external agent which could affect particular proteins of the somatic cells be able also to influence the homologous elements in the germ-cells?

Inasmuch as I have already twice reported to this Society on the work[4] of Dr. Smith and myself with fowl-serum immunized to rabbit-lens by means of which, through injections into pregnant rabbits, we succeeded in obtaining defective-eyed young, I shall not again relate the details. The most interesting thing about the experiment was the fact that the eye-defects were transmissible to subsequent generations, and inasmuch as the condition

[3] *Cf.* Child, "Individuality in Organisms"; also "Origin and Development of the Nervous System."

[4] *Jour. Exp. Zool.*, 31, 2, 1920. Am. Nat., LV, 1921.

could be passed down through the male line alone it is evident that it is based on changes in the germ-cells.

In later experiments we obtained similarly defective young by injecting rabbit-lens into pregnant rabbits, although we secured this result only after repeated trials and in the young of but one female. Our belief is that the cytolytic serum not only attacked the newly forming fetal lens, but also its representatives in some of the germ-cells of the fetus. This implies, of course, that there is a sufficient thread of chemical identity between the two to render them both susceptible to the same specific influence.

For such serumal effects to be of significance in evolution, however, the antibody or other factor operative on a given tissue-protein would have to be one that could arise directly in the organism itself. But since it is known that animals will develop anaphylaxis against tissues of their own species, and that a rabbit can be made to build spermatotoxins against its own spermatozoa, it is reasonable to suppose that if an animal's own tissues became displaced, injured or otherwise modified, they might cause the production of antibodies. And these, carried by the circulating fluids of the body into the gonads, would have opportunity to influence such protein-complexes there as were similar to those in the tissues which served as antigens. Nor need the germinal and somatic elements in question be identical in constitution, for it is known that while an antibody against a particular tissue shows its highest degree of specificity only against that tissue, nevertheless, it will also react in some degree with other tissues of the same individual. This phenomenon, termed species-specificity, clearly indicates that there is a broad common basis of chemical identity underlying all the tissues of an organism. It is not unreasonable, then, to believe that there is sufficient chemical identity between the proteins of tissue cells and the related proteins of the germ for both to be influenced by the same agents.

To construct a working hypothesis upon the possibilities

before us we might suppose that as long as all tissues are in normal physiological balance no antibody or similar modifying agents are developed. The germ-cells, therefore, maintain the exact constitution they derived from the zygote from which they descended. But with the occurrence of injury, undue stimulation or pronounced change in any part of the body, serological changes would probably be produced in the blood-stream and the germ-cells would then be exposed to possible modifying influences. This would be more likely to happen, of course, if the exposure continued through a long period of time. If the influence were disintegrative or poisonous as the cytolysins or cytotoxins evidently are, then probably degenerative changes would ensue. Such a hypothesis affords, perhaps, a plausible explanation of such deteriorative evolutionary processes as those seen in the formation of vestigial organs. As a concrete illustration, purely hypothetical of course, we may suppose that such a species as the mole in gradually changing to a subterranean existence would meet with frequent injuries to the eyes, and that, as a result of the ensuing inflammatory and suppurative conditions, resorptive influences would be set to work which not only affected the proteins of the eye, but also the related proteins of the germ. Once the degenerative process got to going, it might for a time be based in each new generation upon both the direct chronic irritation to the eye and the parallel changes induced in the germ. Finally, we may suppose that the somatic influence would cease when the eyes became of small size and the eyelids remained permanently sealed, but that the conditions induced in the germ would persist. If such atrophied eyes continued to be resorbed more or less in each generation, however, variation toward still further reduction might continue in the germ. Such a progressive degeneration might possibly be ranked as an instance of regressive orthogenesis.

But what of the progressive aspects of evolution? Can serological reactions be invoked here with any show of reason? One great difficulty in dealing with progressive

variation is that we know almost nothing about the chemical and physical factors which underly growth, hypertrophy, hyperplasia, metaplasia, or other changes in somatic tissues due to changed internal relations or to unusual environmental stimuli. If we only knew, for instance, what happens in even as simple a case as when epidermal cells develop into a callous in response to undue pressure or friction, we might have a clue as to how, also, constructive changes might occur in germ-cells; but we have no such knowledge.

Certain types of tissue-overgrowth[5] in which there is increase in the size of the tissue-elements (*hypertrophy*) or in the number of such elements (*hyperplasia*) are interesting in this connection. For example, increased strain in bone leads to increased growth of bony tissue, or excessive exercise leads to overdevelopment of certain muscles. In such cases an increased demand on the nutritive stream caused by unusual katabolism results in a physiological hypertrophy. That is, an excessive synthesis of certain types of proteins is set up. Does the impetus to such extra synthesis extend also to the related, though unstimulated, tissues? I know of no evidence bearing directly on this point, although such phenomena as compensatory overgrowth show that there are influences at work outside the immediate tissue itself which are instrumental in inducing the hypertrophy. For example, if one of a pair of organs (lung, kidney, testicle, thyroid) is lacking or is destroyed, the other enlarges in a short time to the size and functional capacity of the pair combined. There is considerable evidence, particularly in the field of pathology, to show that under ordinary conditions the tissue-elements exert a sort of balanced reciprocal restraint, but disturb this and the whole system is more or less deranged until a new equilibrium is established. Since in compensatory adjustments the compensating organ is generally not in direct connection with the one which is missing or disturbed, it seems probable that the agent which incites the hyper-

[5] *Cf.* any General Pathology.

trophy is carried by the blood, although the possible influence of the nervous system must also be reckoned with in higher animals. And if there is such a serum-borne agent in the case of compensatory hypertrophy, may there not also be one in that of the ordinary physiological hypertrophy of the exercised muscle or stressed bone? If so, we must keep our minds open to the possibility that it may also stimulate the germinal prototypes of such proteins to additive functioning. For if we had but a single side-chain in common between a protein of somatic tissue and a protein of the germ, anything that could affect one might well be expected to affect the other.

Again, mechanical stimuli, if not too severe, and various irritative substances in amounts sufficiently small not to be destructive or poisonous to the tissues, may stimulate cells to overgrowth. Very small amounts of arsenic or phosphorus, for example, may thus affect the kidneys and liver, and minute doses of phosphorus may cause increased growth of bone. In such cases also it is probable that serological changes are involved. This is almost certainly true in cases of vicarious overgrowth, where an organ supposedly of related function takes over wholly or in part the work of another tissue. An example of this is seen in the enlargement of the pituitary gland when the thyroid is atrophied or removed, or the compensatory enlargement of the hemolymph glands and bone-marrow following removal of the spleen. Still further may be cited the phenomena of metaplasia, in which, through modification of function and nutrition, specialized tissues develop from cells which normally produce tissue of another order. It sometimes happens, for example, that the choroid coat of a severely injured eye, after the lapse of considerable period of time, will develop a layer of true bone. In fact, metaplastic formation of bone is common in many tissues. Such facts show that many, if not all, tissue-cells have the capacity to form very different kinds of tissue in different environments, and suggest that they retain all the inheren-

cies of the germ. They are what they are somatically because of the special restrictions or excitations of their particular situation in the organism. But if these highly specialized tissue-cells can be so stimulated as to form an entirely different type of tissue, may not such stimulative influences invade even the germ-cell with modifying effects?

Lastly, there are the endocrinal secretions to be reckoned with. Since they are at present popular subjects of research and are constantly being alluded to and discussed in the biological literature of the day, I need not review the field. It is evident that in them we have circulating through the body a series of powerful substances capable of producing profound effects in any or all parts of the body. Through them, apparently, various organs effect reciprocal stimulations and the tissue-complexes of the entire body are maintained in a state of general physiological equilibrium. Both clinical and experimental evidence reveals that hypertrophy or atrophy of an endocrine gland may be followed by marked alterations of structure or function in one or more regions of the body. Thus the cretinism or the myxœdematous condition which results from removal of the thyroid or arrest in its function, or the symptoms resembling exophthalmic goiter following hyperthyroidism are familiar examples. The remarkable overgrowth of the bones of the extremities and head known as acromegaly—morbid giantism—associated with enlargement of the pituitary body is another. Also the relations of the gonads to the secondary sexual characters are well known, as is that of the fetus to the normal hypertrophy of the mammary glands in pregnancy.

Since hypertrophy or atrophy of an endocrine may produce deep-seated permanent changes in various tissues of an organism, I would again point out the possibility that the germinal homologues of the proteins of such tissues, if such there be, might likewise be permanently modified, and that if for some reason there came a constant inherited increase or diminution of an endocrine

gland, or an environmental modification of it generation after generation, we might have in its waxing or waning output the excitant necessary for germinal changes which become outwardly expressed as a series of orthogenetic changes. That this suggestion of endocrinal influence on the germ is not so far fetched as would appear at first sight, is evident, I think, when we recall that certain of the conditions which can be induced in individuals by experimental or pathological endocrinal upsets are known to occur also as congenital defects which are inheritable. For example, short-fingeredness (brachydactyly) may be induced after birth by too much pituitary secretion, but such a condition is also well known as a congenital defect which is hereditary. In the latter case, is it more reasonable to suppose that the short-fingeredness, could we trace it back into the germ, is really represented by a factor that has to do primarily with the finger or with a factor directly concerned in some way with the pituitary body? And did hypertrophy of the pituitary body originally induce the heritable type of brachydactyly? We do not know, but the parallelism of the two conditions, it seems to me is highly suggestive.

And let us glance for a minute at one of the well-known studies on orthogenesis; that of Ruthven[6] on the variations of scutellation in the garter snakes. In his own words:

> . . . it seems to me that the most tenable hypothesis of the evolution of the genus *Thamnophis* is that it originated and became differentiated into four main groups in northern Mexico. From this region the groups radiated in all directions, but principally to the northward, and wherever they entered different regions the changed environmental conditions acted as an unfavorable stimulus which retarded growth, and differentiated the groups into dwarfed forms.

And in another place he generalizes as follows:

> (1) That the maximum scutellation and size in the genus *Thamnophis* occurs in the center of dispersal, and the forms that have been produced in the history of its migration have been formed principally by dwarfing and by a reduction in scutellation; (2) that the variation in the number of scales in the different series is

[6] Bull. 61, U. S. Nat. Mus., 1908.

definite and not promiscuous, and is correlated in a remarkable degree with changes in the environment.

Have we not here a condition strikingly like what we should expect to find if some factor or factors, external or internal, were operating in such a way as to lessen the output of some endocrinal secretion concerned in growth or the determination of size? This is at least a possibility worthy of consideration.

In closing may I say that in what I have put before you I do not pretend to have supplied the established facts necessary for founding a scientific theory. The discussion is largely a series of suggestions, a mere working scheme which takes into account various phenomena that appear to be related, and which in their present states of disclosure seem to lend themselves to some such interpretation as I have tried to give. It is presented because, in my estimation, it suggests a line of thought we may well entertain when we are wrestling with our several problems of genetics, variation and evolution. For if it can be clearly established that any one of the serological influences can reach specifically from soma to germ then it becomes a plausible hypothesis that many of them do. If a changed or changing external or internal environment causes a long continued physiological stress of certain parts, then as long as this stress is accompanied by changed conditions of the circulating fluids of the body, so long also will the germ-cells be exposed to these influences. If they are such as to induce variations in definite directions, orthogenesis must be the outcome.

And if serological influences play an important part in adaptive somatic changes, such as adaptive hypertrophies—or for that matter, adaptive atrophies—then we have the way open to conceive of how adaptive germinal changes may likewise be the outcome of these same influences.

It is a noteworthy fact that in the geological past whenever conditions suitable for new types of existence occurred, new forms of life well adapted to the conditions appeared. This has happened not only once, but

repeatedly. And since satisfactory adjustment to the new conditions must mean not only one, but many favorable and interrelated variations, it seems almost incredible that the adaptedness characteristic of the organisms in question was attained merely through the operation of natural selection generation after generation on assemblages of purely accidental mutations. Palæontologists tell us that times of marked evolutionary change have coincided with periods of great geological change—extremes of temperature, moisture or drought, or fairly rapid fluctuation between such extremes. And while such conditions would undoubtedly favor a maximal operation of natural selection, it is well to remember also that the severe strains of somatic adjustment forced upon organisms existing at the time would doubtless result in a maximal sweep of serological influences through the sorely pressed body.

Although I have emphasized one side of the problem of variation, I am not unmindful of the remarkable stability of the germinal protoplasm as we see it expressed in organisms to-day. It is obvious that not every minor or temporary alteration in somatic mechanism is reflected in the germ to any measurable extent. Since probably no two living things of any kind are equally susceptible to external influence, individual germ-cells doubtless vary in susceptibility, possibly even the same germ-cell would respond differently at different stages of maturity. It is not unreasonable to believe, moreover, that only a few out of many germ-cells might be sufficiently affected to make a perceptible difference. I have already expressed the opinion[7] elsewhere that "no one to-day, qualified by his knowledge of embryology and genetics to the right of an opinion, would, I think, deny that the new organism is in the main the expression of what was in the germ-line, rather than of what it got directly from the body . . . ." But we know that the germ does change from time to time and it seems to my mind not illogical to suppose that at least some of the changes are specifically related to changes in the soma.

[7] Am. Nat., LV, Mar.–Apr., 1921.

ORTHOGENESIS AS OBSERVED FROM PALEONTOLOGICAL EVIDENCE BEGINNING IN THE YEAR 1889[1]

DR. HENRY FAIRFIELD OSBORN

American Museum of Natural History

1. The Origin of Species

The Origin of Species is now clearly understood in the hard parts of invertebrates and of vertebrates, and there is little to be added as to the *modes of mechanical evolution.* No chances or experiments are tried by Nature. The process is continuous, adaptive, mechanically perfect in every Mutation of Waagen. As shown in actual observations by all close students of vertebrate and invertebrate morphology during the last fifty-two years, and as summed up in the remarkable contribution of D'Arcy Wentworth Thompson (1917) on "Growth and Form," animal mechanisms compete with each other in close analogy to humanly made machines—automobiles, typewriters, aeroplanes. Consequently, while Nature is constantly standardizing her machines through individual competition and producing flocks of birds and shoals of fishes which are so precisely alike that animals of the same age, sex, environment and heredity *show no perceptible variation,* she is also frequently substituting more perfect and more adaptable machines and discarding older and less adaptable ones, exactly as man is doing in the case of his automobiles, his typewriters, and his aeroplanes. Thus the naturalist and the paleontolog-

[1] Illustrated by twelve lantern slides exhibiting mutations of *Ammonites*, of *Spirifer*, of *Paludina;* rectigradations of the grinding teeth of lemuroid Primates; evolution of proportion from the rhynchocephalian type of *Hatteria* to the dinosaurs and birds of the genera *Deinodon*, *Struthiomimus*, and *Diatryma.*

ist are alike impressed with the incessant action of Natural Selection on animal mechanisms and with the new testimonials to this aspect of Darwin's great principle.

When it comes to the *origins* either of new characters or of new proportions quite different is the attitude of observers of mechanical evolution; no evidence whatever has been forthcoming from the same fifty-two years of close observation and research as to the *causes* of origin, at the same time the *modes* of origin of all mechanical characters are indubitably orthogenetic.

To further clarify the bearing of palæontology on orthogenesis, I desire to point out that all visible mechanical evolution goes hand in hand with invisible physicochemical evolution; and that there are steps in evolution which are primarily physical, others which are primarily chemical, others which are primarily mechanical. Therefore the experimental botanist, zoologist, biochemist, biophysicist, or geneticist, has the opportunity to win immortal fame by discovering the causes of mechanical evolution.

Meanwhile the palæontologist enjoys the entirely unique position of being the only competent observer of the Origin of Species so far as specific characters are recorded in the hard parts of animals and the relatively few soft parts which are preserved in a fossil condition.

2. Orthogenetic Origin of New Characters

All agree that sound induction either as to the origin of new characters or their transformation is an exceedingly difficult matter. It has taken me thirty-three years of uninterrupted observation in many groups of mammals and reptiles to reach the conclusion that the origin of new characters is invariably orthogenetic.

In 1889 I first observed (Osborn, 1889.46) that new cusps originate on the grinding teeth of Eocene Primates, now recognized as lemuroids, in a definite and adaptive manner from minute shadowy beginnings which are mechanically adjusted to similar minute shadowy

beginnings of opposing cusps in the other jaw; whereby there evolves a continuous reciprocal mechanism not dissimilar to the reciprocal services of the Yale key and the Yale lock. The evolution of the key below proceeds with the evolution of the lock above. The process does not go very far in the Primates, but in the purely herbivorous ungulates, like the horse and the elephant, the reciprocal grinding mechanism reaches a degree of complexity to which the most intricate lock and key devised by man present but a feeble parallel. Every mechanical device in the upper grinding teeth, adapted to the fine comminution of grasses, is *reversed* in the lower grinding teeth, on the principle of mechanical action and reaction; nowhere in nature is reciprocal mechanical co-adaptation more perfectly evolved than in the upper and lower grinding teeth of mammals.

Between 1889 (Osborn, 1890.47) and 1891 (Osborn, 1891.53) I made what I now believe to be an *unsound induction* from this evidence that this continuous mechanical origin tended to support the Lamarckian theory of the inheritance of adaptive reactions. I first termed the orthogenetic process "definite variation"; later I termed it "progressively adaptive variation"; by the year 1908 I realized that these new adaptively arising tooth elements were not variations in Darwin's sense at all, and I applied to them the distinctive term *rectigradations* (Osborn, 1908.314). In the meantime I abandoned the Lamarckian explanation and in 1895 (Osborn, 1895.97) I started out upon a *search for the unknown factors of evolution,* a search in which I am still busily occupied.

To return to the difficulty of making sound inductions as to the origin of new characters in hard parts, in 1889 I opened a long correspondence with the leading exponent of Darwinism in Great Britain, Edward B. Poulton, who admitted the evidence but interpreted the facts in the Darwinian way, namely, as the selection of mechanical successes from non-observed mechanical failures. It is a good thing to have a number of skeptical friends

about; it sharpens your powers of observation and makes you much more cautious about your inductions. My original observations on the Primates required corroboration, and this I have sought through the observation of the origin of new characters in many other kinds of mammals traced in their evolution over very long periods of time, especially the horses, the rhinoceroses, and recently the proboscideans, but most profoundly and exhaustively the titanotheres, an extinct family remotely related to the horses, which I have studied monographically for twenty-one years.[1]

Even by trying to keep an absolutely open eye and mind, entirely uninfluenced by any theory, or preconception, or opinion, I have been unable to find a single exception among these many different kinds of mammals to the observations made on the Primates in 1888 and 1889; not a single new organ is observed to arise fortuitously or indefinitely; it always arises gradually, continuously, and adaptively from its minute shadowy beginnings. This continuous reciprocal, mechanical coadaptation seems to be an established fact in evolution, and is established most strongly where explanation or search for causes seems to be most difficult.

I am not enthusiastic about the adoption of the term orthogenesis, admirably significant as it is in its Greek derivation, first, because Eimer connected it with Lamarck's and Buffon's principles of inheritance of acquired modifications, and, second, because it does injustice to the first great observer of direct adaptive origins in nature, namely, the German palæontologist Wilhelm Heinrich Waagen, whose observations in 1869 laid the foundation of all subsequent work both among the in-

[1] Osborn, H. F., "The Titanotheres of Ancient Wyoming, Dakota, and Nebraska. Life and Geography of the Central Rocky Mountain Region in Eocene and Oligocene Times. Evolution of the Titanothere Family. The Causes of Development and Extinction of Mammals," *U. S. Geol. Survey Monograph No. 55.* [Unpublished.] Completed for the Survey June 30, 1920. This monograph is the most complete and exhaustive analysis that has thus far been made of the evolution of any family of organisms.

vertebrates and the vertebrates. To the best of my knowledge *he was the first naturalist to observe how new species actually arise in nature.* Compare Waagen's description (1869) of the genesis of new characters in the shells of cephalopods (*Ammonites subradiatus*) with those which Osborn (1889–1921) has observed in the teeth:

"*Thus the species if considered as such may be conceived and considered as a species, but in contrast with earlier or later forms* [*i.e.*, ancestors or descendants] *as a mutation. Now as regards the value of these above-defined conceptions, variety and mutation, on closer consideration a quite decided difference in value becomes apparent. The former conception* [variety], *in the highest degree variable, appears to be of small systematic value; while the latter* [mutation], *although in minute characters, is highly constant, always surely recognizable; on which account far greater weight must be put upon Mutations, they ought to be very precisely denoted and held fast to with great persistence.*"

Twenty years later the German palæontologist Melchior Neumayr observed this process of continuous development, generation after generation, in a certain definite direction for which he proposed the term "*Mutationsrichtung.*" Thus the "mutation of Waagen" arises continuously through the inner working or tendency, the "*Mutationsrichtung*" of Neumayr.

It was not until 1894 that William B. Scott brought Waagen's term "Mutation" to the notice of vertebrate palæontologists in this country, in antithesis to Darwin's term Variation. Waagen's "Mutation" means one thing, Darwin's "Variation" means quite another, as pointed out by Scott above. The term Mutation in Waagen's sense is now widely but not universally used by palæontologists to designate intermediate gradations of minor taxonomic rank which are observed in ascending or descending series of animals to connect the larger stages of evolution which we call Species. As an ele-

mentary species a "Mutation" of Waagen is comparable to a "Mutant" of De Vries in external appearance, but not in mode of origin, because one arises through a continuous *"Mutationsrichtung,"* while the other arises through accidental germinal saltation. To my mind the continuous or discontinuous mode of origin either of a "mutation" or of a "mutant" is of small account as compared with the fortuitous or orthogenetic nature of the impulse in the germplasm which gives rise to it.[2]

So far as I know all observers of the hard parts of extinct animals, whether vertebrate or invertebrate, confirm this classic observation of Waagen, and many in this special field of observation also confirm the *"Mutationsrichtung"* of Neumayr. So far as I personally have observed, this principle of "Mutationsrichtung" is especially dominant in the origins of characters; here at least other interpretations are not applicable; there is no question of Selection between two alternatives, adaptive and inadaptive, because the inadaptive does not occur, the whole process is adaptive and the difference between two organisms is the rapidity and direction with which the *"Mutationsrichtung"* is acting. This is the same in the hard parts of the molluscs *Ammonites, Paludina,* and *Planorbis,* as it is in the mammals *Equus, Rhinoceros,* and *Elephas.*

3. The Origin of New Proportions

In the evolution of proportions, that is, proportions in the different parts of skeleton and skull as in *Sphenodon, Deinodon, Struthiomimus, Diatryma,* it appears *probable* that Selection may be constantly working on all adaptive fluctuations of proportion in connection with ontogenetic modifications in proportion which are also adaptive, as in the classic case cited by both Darwin and Lamarck of the length of the neck of the giraffe.

[2] F. A. Bather in 1905 (*Proc. Geol. Soc.,* Vol. 61, pp. lxxii–lxxiii) most clearly elucidated Waagen's conception of the Formenreihe and of the Mutation in *Ammonites.*

It has been demonstrated experimentally that the limb proportions in the brief life of a dog may be modified from the cursorial to the saltatorial type by amputating the fore limb. This is a process of reciprocal Modification and Selection which Osborn, Baldwin, and Morgan term Organic or Coincident Selection. I have devoted an immense amount of study to the causes of the evolution of proportion and have come to the conclusion that orthogenesis in the evolution of proportion may be apparent rather than real. In other words, whenever a character assumes a survival or elimination value, it may develop very rapidly through the selection of fluctuations in the right direction and may result in apparent but not real orthogenesis.

4. Summary as to Orthogenesis

The visible "mutation of Waagen," or "definite variation" or "rectigradation" of Osborn appears to depend on the "Mutationsrichtung" in the germ-plasm. The final question in my mind, as in yours, must be, if such a "*Mutationsrichtung*" exists, is it the "internal perfecting tendency," is it the "vitalism," is it the "creative evolution" which the majority of biologists are so skeptical about?

I observe that it is not. I observe that while the "*Mutationsrichtung*" is a real process, it differs from any kind of internal perfecting tendency in the fact that it consists in an adaptive reaction to the particular environment in which a series of organisms is placed, or to which it migrates. For example, the internal perfecting tendency to arboreal life does not manifest itself when the animal seeks an aquatic life. Conversely, aquatic adaptations are not constantly springing up among arboreal mammals. Observations on fossil forms have led to Dollo's remarkable generalization regarding "alternate adaptation," which renders any form of internal perfecting tendency in any predetermined direction inadmissible.

Summary of Observations.—In the hard parts of animals orthogenesis is observed both in the origin of new adaptive characters and in the evolution of proportions. (1) The induction as to cause may be different in the two cases. (2) In the origin of new adaptive characters orthogenesis is attributable to definite germinal tendencies. (3) The origin of changes of proportion which are subject to *modification* may be partly attributable to Organic Selection. (4) There is positive disproof of an internal perfecting tendency (Vitalism) in either the origin of new characters or the origin of proportions. (5) There are certain changes of length and breadth proportion both in the shells of invertebrates and the skulls of vertebrates which can not be explained by Organic Selection. (6) There is very strong support in fossil series for Selection incessantly acting on all characters of survival or elimination value.

The above six principles are those which I have derived from forty years of continuous observation; they are actual modes of the mechanical evolution of new species for which we have no theoretic explanation, unless it be that of Organic Selection in the single case above noted.

Summary of Opinions.—I may add as a matter of personal opinion and hypothesis three points: *first,* that we are as remote from adequate explanation of the nature and *causes of mechanical evolution* of the hard parts of animals as we were when Aristotle first speculated on this subject three hundred years B.C.; *second,* that the chief outlook for experiment is in the domain of physics; *third,* that the explanation, if ever it is to be found, is to be along the lines of four systems of energy (= Tetraplasy, Tetrakinesis, Osborn) which surround the origin and development of every character in every organism; *fourth,* I think it is possible that we may never fathom all the causes of mechanical evolution or of the origin of new mechanical characters, but shall have to remain content with observing the modes of mechanical evolution,

just as embryologists and geneticists are observing the modes of development, from the fertilized ovum to the mature individual, without in the least understanding either the cause or the nature of the process of development which goes on under their eyes every day.

In conclusion, it is the great biological achievement of the last half century that palæontologists have discovered *how* new characters and new species originate. It may be the achievement of the experimental biologists during the next half century to explain *why* new characters and new species originate.

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THE EFFECTS OF ENVIRONMENT ON ANIMALS[1]

PROFESSOR A. S. PEARSE

University of Wisconsin

As Henderson[2] has pointed out, the environment on the surface of the earth is suited to, and largely responsible for, the existence of living organisms. After an organism comes into existence, it strives to live in harmony with its immediate environment. An organism is a "system of activities"[3] which devotes its energies primarily to three functions: (1) capturing energy for and releasing energy from its own system, (2) protecting its system from injury, and (3) producing other systems of activities similar to itself. If possible an organism reacts with its environment in such a way that its system continues to exist and carry on its three primary functions. It is limited in its responses to a particular behavior pattern, inherited from the system from which it came, but in general it reacts in such a way toward its environment that it selects by trial the optimum conditions for its own existence. In other words, an organism generally responds in an adaptive way and selects the best environment that it can. If the behavior patterns of certain systems, similar or dissimilar, are well suited to a particular environment, such systems often are "successful." They may take possession of the environment, perhaps exterminating other systems, and, thus demonstrating their "fitness," constitute what ecologists call a climax formation. Every organism in such a group must remain a system of activities and must make continual physiological adjustments to keep in harmony with the environment, or it can not continue to exist. Each organism assumes a

1 An address before the Geographical Society, University of Wisconsin, January 11, 1922.

2 "The Order of Nature," Cambridge, 1917.

3 This definition is not intended to exclude the possibility that an organism may be more than matter and energy. It may contain an entelechy or something similar, but as yet there is no scientific proof that it does. The limitation, and value, of science is that it must always deal with facts.

particular internal pattern that consists of a graded series of metabolic activities which (*de* Child) is a direct response to stimuli received from the environment.

In responding to environment plants and animals show fundamental similarity. Many plants adjust themselves to their surroundings by assuming the form that best suits them to the particular space in which they happen to take up a sessile life, and many animals secure a place which is suited to their system of activities by moving about until they find it. This difference between plants and animals is largely due to the fact that the former usually are able to subsist on inorganic foods, while the latter require organic substances as a basis for their metabolic activities. However, animals often respond to the environment by assuming a particular growth form, and plants have many motile systems of activities that find favorable environments through active or passive migrations. Being trained as a zoologist and knowing little of the activities of plants, I gladly take the task assigned to me—"to discuss the effects of environment on animals"—but I can not refrain from expressing my opinion, that there is no essential distinction in this connection between the two great kingdoms of life.

Animals are continually active and must continually react with the environment. Alcock[4] said, "the three great exigencies: to find something to eat, to avoid being one's self eaten, and to disseminate one's species, give rise to a perpetual struggle in which the fittest are successful." The environment furnishes matter and energy to maintain the activities of each system and a considerable quantity of both is neccessary. A silkworm during its short life eats food amounting to 86,000 times its own weight at the time of hatching. Animals take the most diverse mateials from the environment and use them to build substance or furnish energy. The clothes moth flourishes on a diet of wool, which consists entirely of keratin. From this almost pure, and to most animals wholly indigestible, pro-

[4] "A Naturalist in Indian Seas." London, 1902.

tein substance the moth makes carbohydrate, fat, and water to supply the needs of its system. The bee moth subsists on bee comb, which contains less than one per cent. of protein and a large amount of rather insoluble wax. Ants not only acquire food from the environment, but give up what they have already swallowed to their fellows, even when they are hungry themselves. In this case the "system" of the colony is more important than that of the individual.

In order to keep their systems of activities intact, animals have adopted many means to escape dangers. There are lurking enemies, physical changes, accidents, insidious parasites to be met or avoided continually. A walking-stick spends nine-tenths of its life in a "perfectly quiescent" state, depending on being overlooked by hungry enemies. A house fly escapes through endless agility. A rotifer avoids drying up by secreting a cyst about itself, and may remain dormant for years. Many animals are able to change the usual rate of their metabolic activities in response to changes in temperature and pass cold periods in a hibernating state.

Animals, before all things, use the means they possess in order to perpetuate their particular systems. New individuals must continually be started on new life cycles and such recreations involve reorganizations of systems, changes in metabolism, and various responses by organisms to the environment. Such qualities as odors, colors, and songs may be very important for the survival of a race. A male moth will migrate a mile or more to find a mate—attracted by her odor. The daily routine of seeking and escaping dangers is often neglected by animals when the survival of their race is concerned. Greedy penguins allow any youngster that comes to feed from their crops. An adult bull seal takes no food from May to August, but devotes all his energies to the defense of his rookery. The male gaff-tops'l catfish takes the eggs from his mate and carries them in his mouth for ninety days—denying himself food in order that his offspring

may survive. The little spider that spins a cocoon under stones guards her treasure with watchful care and, if she is compelled to leave her cocoon, spins a ground line as she runs in order that she may return without delay. A male spider dances, postures, and uses all his arts to secure a mate. As soon as he has mated, Nature usually sacrifices his life to his offspring—for his hard-earned mate devours him if she can.

Thus it is wherever one considers animals. There is adjustment, frequently of a very specialized type, to environment. The wonder of it all is the degree of adaptation that animals show. In speaking of food relations Semper[5] said, "there is scarcely a constituent of the earth's crust, whether on land or in water—not an animal nor a plant, whether living, dead, or even in decomposition—which does not afford nourishment to some living animal." The first more or less self-evident generalization justified by this discussion may be stated as follows: *Animals are adapted to the environment.*

That animals are adapted, probably no one disputes, but there has been much controversy as to the means by which they have become adapted. There appear to be three effects that it is possible for the environment to produce in animals: (1) a direct transformation or modification of the living system of activities, (2) the destruction of systems unsuited to the environment and the "survival of the fittest," and (3) the migration of systems from unfavorable to favorable environments.

Animals are modified by external changes and may even take on different forms to suit different environments. Sponges and corals growing in deep water usually have a branching form; the same species in shallow water form flat, encrusting growths. The brine-shrimp, *Artemia salina,* is a classical instance of an animal that has many forms, and these are rather closely correlated with the salinity of the water in which it lives. Sumner[6] and Shel-

5 "Animal Life as Affected by the Natural Conditions of Existence," N. Y., 1881.

6 Bull. U. S. Bureau of Fisheries, 1910.

ford,[7] working independently, have shown that very slight structural differences that distinguish closely related species of amphipods and tiger-beetles are correlated with distinct habitat preferences. The structure and physiology of animals are modified by environment—the structures and activities of the systems are changed. Different species may possess almost identical structures, but show specificities of behavior in relation to environment.

Darwin made much of the struggle for existence among animals, pointing out that many species hold their places on the earth through wide dissemination and selective survival. One who has seen the strangler trees gaining a foothold in the tropical forest, the fiddler crabs fighting to hold a favorable place on an ocean beach, or the oysters in an overplanted area striving to survive, can not doubt that there is such a struggle. More animals are produced than can find a place to exist, and in general those survive that are best suited to the environment that is available.

Animals are not always obliged to adjust themselves to the environment or struggle for a favorable place to live in it. They migrate from situations where their systems can not well carry on activities to some spot where conditions are more propitious. In such migrations animals have very definite relations to the environment. They are limited by their reaction pattern to certain habitats; they must disperse from their "centers of origin" through "highways," and are prohibited from migration into certain regions called "barriers." Barriers are areas where certain environmental factors vary beyond the limit of toleration for a species. A "center of origin" as usually understood by geographers, may be the real place of origin of a species or it may merely represent the locality where the most environmental factors are favorable. In general a uniform environment covering a wide range of territory permits the species suited to such an environment to have a wide geographic range.

[7] *Biol. Bull.*, 1911.

Variable species usually have wider ranges than unvarying, because they can adapt themselves to more environmental variations.

A second generalization is appropriate here: *Animals become adapted to environment by* (1) *transformation,* (2) *selective survival from an overpopulated condition,* (3) *migration from unfavorable to favorable situations.*

It will be profitable now to examine two or three typical associations in order to study animals in action with the environment. If, in this connection, one thinks over the great responses that animals have made to environment in the past, he will probably conclude that the greatest habitat change has been that from water to land. It is generally supposed that life first appeared in water. As a habitat, water has certain inherent advantages—the chief of which is perhaps the slowness with which temperatures change. It also has certain disadvantages, the most important of which are probably the variability of its dissolved gases (the higher the temperature, the less gas can be held in solution) and its general solving power, which makes it a transporting medium for all sorts of substances, some of which are poisonous. All animals require a more or less constant supply of water and of oxygen for metabolic processes. When animals forsook the water for land habitats, they gave up surety of water supply and conditions of reasonable thermal stability. What did they get in return? Apparently nothing but a stable gaseous condition for respiratory needs. The danger of desiccation and the wide variations of temperatures incident to land life were apparently compensated for by this gaseous stability. Yet the attractions of the water have at times led many animals, like the aquatic insects, that had become adjusted to life on land to revert to aquatic habitats. In the past races have doubtless many times become adapted by transformation, selection or migration on account of the advantages or disadvantages of one of two habits.

If one walks along a rocky shore, where the ocean

waves and tides sweep, he may be surprised to find an abundant fauna in the " 'tween-tide " zone. The moving water, teeming with microscopic organisms, brings an abundance of food to those animals that are able to stand the beating of the waves and the alternate submergence and exposure due to the ebb and flow of the tides. A rocky wall along the sea shore is no place for weaklings. One minute the blistering sun bakes the exposed animals; the next, the rising tide has covered them with cold water. The waves beat ceaselessly. The changing seasons bring ice and torrid heat. How are the animals on these rocky shores responding to the environment? Here one finds a variety of hardy species which, though not closely related genetically, have many characteristics in common. There are sponges, anemones, hydroid colonies, barnacles, mussels, snails, small crustaceans, and a few scavenger crabs. These animals for the most part obtain their food by net fishing or by straining water through their bodies. They are mostly attached firmly to the rocks, and thus withstand the violent movements of the food-laden water. The barnacles, sponges, and hydroids are grown fast; the anemones and snails have sucking discs that enable them to adhere firmly; the crustaceans have claws for attachment and hard armor covering their bodies. Some of these animals are small and can easily hide in crevices; some of those of larger size, like crabs, are able to migrate to other habitats during violent storms. If an animal is attacked, it is advantageous for it to be able to receive stimuli with facility from all directions of the compass, and, as would be expected, many of the animals on rock beaches are radially symmetrical. Radial symmetry has marked advantages for sessile animals, but puts a weighty limitation on psychic development. An animal that is able to perceive stimuli equally well, through equally efficient sets of sense organs that are symmetrically disposed about a central axis, is never able to develop its power of paying attention to any considerable degree. Its simple mind, if such an animal may be said to have

a mind, must attend now to a stimulus received from one side, now to that on another. Such vacillation is not conducive to the development of higher types of mental life through the delegation of psychic authority to one nervous center. The rocky ocean shores, then, put a premium on radial symmetry and thus as an environment tend to foster psychically unprogressive animals. The barnacles, that appear to have come from progressive, bilaterally symmetrical ancestors, have become degraded with the taking on of the sessile life and radial symmetry that suits them so well to wave-beaten shores.

The ebb and flow of ocean tides have a pronounced effect on shore animals. Those species that are not able to survive alternating exposure to the desiccating effects of air of varying temperatures and the activity of violently moving water of rather constant temperature can not exist on rocky shores. This fauna must be resistant, and is so. An anemone can be kept out of water for a week—until it looks like a dried raisin; or kept in a tightly corked bottle for ten days, and when replaced in the ocean appear to be perfectly normal in an hour or two. Such an animal will not readily succumb to the exposure between tides or even to the stagnation likely to occur in a beach pool that is cut off from the ocean during a prolonged period of low water. The barnacles and molluscs on rocky shores are protected by heavy calcareous shells. Flattely[8] has suggested that land animals perhaps arose in the past on ocean beaches as a result of the resistance developed during exposure between tides.

As a whole the environment occurring on rock beaches offers abundant food, but hard conditions for life. The fauna is highly adapted to resist the two important environmental factors—moving water and exposure to variable conditions—and in this adaptation the fauna has incidentally but of necessity become unprogressive and devotes most of its activities (1) to feeding rapidly when the opportunity comes, (2) to resisting, (3) to resting.

[8] *Science Progress*, 1921.

One does not imagine such a fauna as developing, even through countless ages, great appreciation of beauty, or of any of the æsthetic qualities of "higher" animals. The adaptations here are to resist the unfavorable in the evironment, and still live.

If a man walks in a tropical forest, he is amazed at the abundance and variety of the life about him. He may see a certain species of tree in one spot and not encounter another like it for a mile. Meanwhile he has seen a hundred other species of trees. What is the striking environmental factor in this forest? It is life itself! The environment is favorable for so many systems of metabolic activity that hundreds of kinds of animals are ready to live in it—if they can find a place. Is there a struggle; is there adaptation? Nowhere on the earth are these responses to environment more striking. Most of the struggles to live in the forest are competitions with other living systems that are trying to continue to exist. And the adaptations are not often for resisting, for eating, for resting. Think of all the animals in the tropical forest—is there one that is radially symmetrical? Here keen senses are at a premium. Life has always depended upon seeing, hearing, feeling better than something else. Lately it has come to depend upon thinking better than something else. And the climax of adaptation in this tropical forest has been the greatest thinker of the ages.

A third generalization appears to be justified: *Each habitat, representing environment, limits the patterns of the systems of activities that may persist from reactions within it. The type of adaptation is set by the environment.*

Environment has a quality that any system of activities that attempts[9] to live in it must respond to. This is its changefulness. The paleontologists say that environment punishes too much adaptation by changing. I think it is proper to say that the chief cause assigned for the

[9] The writer realizes that "attempts" may be interpreted as teleological —and rejoices in the sinfulness of it. If an organism does anything, it strives to keep on existing. As far as it possesses means, it responds to whatever interferes with living.

dying out of extinct types of animals is "over-adaptation," or better "too much specialization." A system of activities, as represented by an organism, can not depend absolutely on another system of activities, as represented by environment. The organism changes and the environment changes too. If the environment continues for a time in a fairly stable condition, an animal may become adapted to it to such a degree that, if the environment then does change, the animal can not respond enough to continue to live. The wood frogs in the United States breed when the water is at freezing temperatures; frogs, belonging to the same genus as the wood frog, that live in Cuba die when the temperature falls below seven degrees. These frogs are adapted to different environments and those in Cuba will be in greater danger of extinction if there is a prolonged cold period.

There is a general tendency among animals to find success during conditions of stability. Certain arthropods left the water and attained stable respiratory conditions and freedom from water-soluble poisons by going on land. Later, certain of these arthropods again gained a thermally stable environment in the water and continued to enjoy a stable gaseous environment by carrying air into the water with them. When any race of animals attains a stable environment, it may become specialized to it. We see a manifestation of the same type in the psychology of man. It is "human nature" to desire stability—to be free from care and worry; to know where one stands.

On the other hand, continual change is a stimulus to progressive response—in fact, one is tempted to say that lack of change is injurious to living organisms and that changes often stimulate living systems to renewed activity. Payne kept fruit flies continuously in the dark; Calkins and Woodruff maintained protozoans on unvarying culture media. All these investigators agreed that lack of variation in the environment was injurious. This raises a dilemma—on one hand animals tend to become highly adapted (or specialized) when the environment is

stable, and on the other hand a changing environment is a stimulus to progressive changes in organisms. A few animals have lived for ages in a stable environment. Thompson cites the brachiopod, Ligula, as a "supreme instance of static racial inertia." However, most animals must live in environments that change. How do these respond?

It is a matter of common knowledge that animal systems of activities can become adapted to changes in the environment, even when such changes constitute new racial experiences. By taking increasing doses of certain poisons at regular intervals animals develop enough immunity to be able to take daily a dose which in the beginning would have been fatal. If a pigeon is fed nothing but meat the lining of its stomach changes its character and the bird's metabolic activities become adapted to an unusual diet. Many other instances of acclimatization to new conditions might be cited.

Every physiographer knows that earth environments change by succession. Land forms erode and water forms fill up with sediment. Physiographic succession brings about a succession of environments, or habitats. These are successively occupied by different groups of plants and animals and there is thus an ecological succession, which is a succession of species or groups of species. Shelford has worked out excellent examples of ecological succession in the streams and ponds along the shore of Lake Michigan. Pioneer species of animals invade habitats soon after they are formed, and as the habitats change the pioneer species are succeeded by others that are adapted to later stages in physiographic succession. Ecologic succession is a succession of species; animals do not change as the environment changes, but die or migrate to more favorable localities. Animals do not appear to have special means for adapting themselves to such changes.

There are other types of succession, however, to which animals show striking adaptation. The types are all rhythmic (seasonal, monthly and daily) and depend pri-

marily upon the motion of the earth and moon. As the earth makes its annual journey around the sun, the animals of temperate and polar regions, and to a less extent those in the tropics, are subjected to seasonal changes in environment. These changes are related chiefly to temperature, available moisture, and food. Animals generally respond to such environmental variations by adjusting appropriate activities to favorable times. In general winter is a season for resting; spring, for mating and propagation; summer, for feeding and growth; and autumn for fructification. Seasonal succession is a succession of stages in life cycles. The seasonal rhythm has a short enough period to permit animals to become adapted to it. Their systems of activities vary to fit the seasons. Every one is familiar with the seasonal migrations of animals. The arctic tern travels from pole to pole, and thus always lives in sunshine. Many animals do not migrate, but pass the winter in a dormant condition. In the tropics animals frequently æstivate during the annual dry season. Now many of these seasonal responses are certainly due to stimuli received from the environment. The little Daphnias, that live in fresh-water habitats the world over, usually have long helmets in summer and short helmets in winter, but long-helmeted forms can be made to produce short-helmeted offspring in summer by keeping them at low temperatures. In this instance the effective stimulus appears to be thermal in nature. But animals are adapted to seasonal succession beyond merely responding as far as they are able to stimuli that come with rhythmic changes without their bodies. The living system apparently has a rhythm of its own that is adapted to the seasons. Smallwood[10] kept a female dogfish (*Amia calva* Linnæus) in an aquarium, practically without food, for twenty months at rather constant temperature. During this time the fish twice took on its bright nuptial coloration. Another instance of similar nature has come to the notice of the writer. A tame spermophile, *Citellus tridecimlineatus* (Mitchill), was kept in a steam-heated

[10] *Biol. Bull.*, 1916.

house for four years. In the autumn of the first year it became very fat and stored a large quantity of food in its burrow. About December 1, it went into its burrow, closed the opening, and remained underground for 119 days. The following autumn the spermophile behaved in a similar way but remained underground for only 28 days. It did not hibernate during the two years following. This animal had an established seasonal metabolic rhythm that was correlated with seasonal environmental changes, but the rhythm had a physiological basis for it persisted when appropriate environmental stimuli were not present.

The rotation of the moon about the earth introduces certain rhythmic variations into the earth environment to which animals respond in adaptive ways. Such responses are of course not due directly to the moon as such, but to effects of the moon's motion on matter belonging to the earth. The famous Palolo worm and various other marine annelids come from their hiding places to spawn only during certain phases of the moon. In these worms the eggs do not ripen except when the moon is new or full; the internal activities respond to outside changes, chiefly referable to tidal variations, and a physiological rhythm is established.

The earth rotates on its axis and thus the animals on its surface are subjected to alternating light and dark. Animals readily respond to this short-period rhythmical change. Every one is familiar with nocturnal and diurnal animals. They are adapted to rhythmical environmental changes to such a degree that they may keep on responding periodically when the environment does not change. Keeble and Gamble[11] have described an interesting shrimp (*Hippolyte varians*) that has day and night color phases. During the day this shrimp matches the background on which it rests with a high degree of accuracy, assuming quite a variety of colors and patterns. At night it turns green, regardless of its background. When kept continuously in light it undergoes rhythmic color changes at about the time periods that correspond to day and night

[11] *Phil. Trans.*, London, 1904.

for two days; and makes similar changes in the absence of light for about a week. There is a physiological rhythm that corresponds to periodic environmental changes.

A fourth generalization must again relate chiefly to adaptation—*Though animals possess considerable power of adjustment to new or changed factors in their environment, they apparently do not usually become adapted as species to physiographic changes, but are eliminated by the variation of factors beyond their limit of toleration. One species or group of species succeeds another during physiographic succession. However, animals do respond in an adaptive way to rhythmical daily, monthly and seasonal successions.* Some animals show adaptive responses to rhythmical environmental changes only once during their life cycle. Salmon, for example, do not migrate up rivers to spawn until they have reached a certain age. *Animals apparently become most specialized, or adapted to particular environments, when conditions are most stable. Even the striking instances of adaptations to rhythms show this tendency of adaptation to attain stability—in this case a regularly changing stability.*

Environmental changes have been important in their effects on the evolution of animals. In this paper it has been shown that living systems of activities are adapted to the environment; that they respond to the environment by transformation, selective survival, or migration; that each habitat limits the patterns of the systems that exist within it; and, that, though adaptation to environment may permit precise adjustment to rhythmical changes extending over considerable periods, and though animals generally become most specialized when conditions are most stable, there is no evidence that living systems are caused to change from one species to another by the transformations of habitats due to physiographic succession. The pattern of evolution is set by environment, but there is little or no evidence that changing environment causes adaptive variations of such a degree that new species are produced. Animals adapt them-

selves to environment by changing their systems of activities, but such responses are apparently limited in extent to the inherent possibilities of variation already within the system. Animals have great powers of adaptation to environment, but are not fundamentlly changed by it. Environment permits evolution and controls its course, but does not appear to cause it. If variations fit environment, they are adaptive; if they do not, systems cease to exist. Environment does not appear to cause variation. The living mechanism still holds the mystery of variation within itself. Until there is conclusive evidence, this one great remaining problem of evolution can not be solved. Yet, notwithstanding this lack of evidence, there are still those who belive the environment does cause evolution—though their only foundation for such belief is what Bergson calls "intuition." Until there is proof, science, if it would be scientific, must keep in mind that these "faithful" believers may be right, and be content to wait, perhaps a hundred thousand years—for evidence.

SUMMARY

1. Animals are systems of activities that are adapted to environment.

2. Animals become adapted to the environment by transformation, selective survival, migration.

3. Each habitat limits the patterns of systems of activities that may result from reactions within it. The type of adaptation is set by the environment.

4. Though animals possess considerable power of adjustment to changes in environment, there is no evidence that they became adapted as species to slow changes due to physiographic succession. They do respond to rhythmical daily, monthly, and seasonal changes in an adaptive way. Animals appear to become most specialized, or adapted to particular environments, when conditions are most stable.

5. Environment permits and directs evolution, but does not appear to cause it by forcing the acquirement of new characters.

A SUMMARY OF THE FOOD HABITS OF NORTH AMERICAN COLEOPTERA

HARRY B. WEISS

New Jersey Department of Agriculture

The Coleoptera or beetles contain a very large number of species and show a great diversity of habits. Most of them are terrestrial and they live under almost all conditions where insect life is possible. The economic status of this group of insects is important. To the Coleoptera belong some of our most pernicious agricultural pests as, for example, the cotton boll weevil, which has caused such ruin in the cotton belt, the Colorado potato beetle with its familiar destructive activities and various other species which attack forests and field crops with varying degrees of intensity. However, many species of Coleoptera are engaged in useful activities and it is the purpose of this paper to summarize briefly, and in a very general way, the food habits of the families in this order.

For the purpose of convenience in handling and for the sake of simplicity, the families have been grouped into a few important classes and the placing of each family was based mainly on the predominating larval activities of its members. In some families considerable variation occurs in the food habits of the different species. For instance, in the *Scarabæidæ,* some are destructive to green vegetation and others thrive on vegetable decay. On the whole, however, their activities are saprophytic and for this reason the entire family was placed in the group Saprophaga. The *Staphylinidæ* were placed in this group also, although this family contains members which live in fungi, in animal and vegetable decay, in the nests of ants and some which are predatory. In quite a few of the families, the activities of the species are practically identical.

The classes into which the families are grouped are as follows: Phytophaga, Saprophaga and Harpactophaga. In addition to these three important ones, the species attacking mammals and those whose family habits are

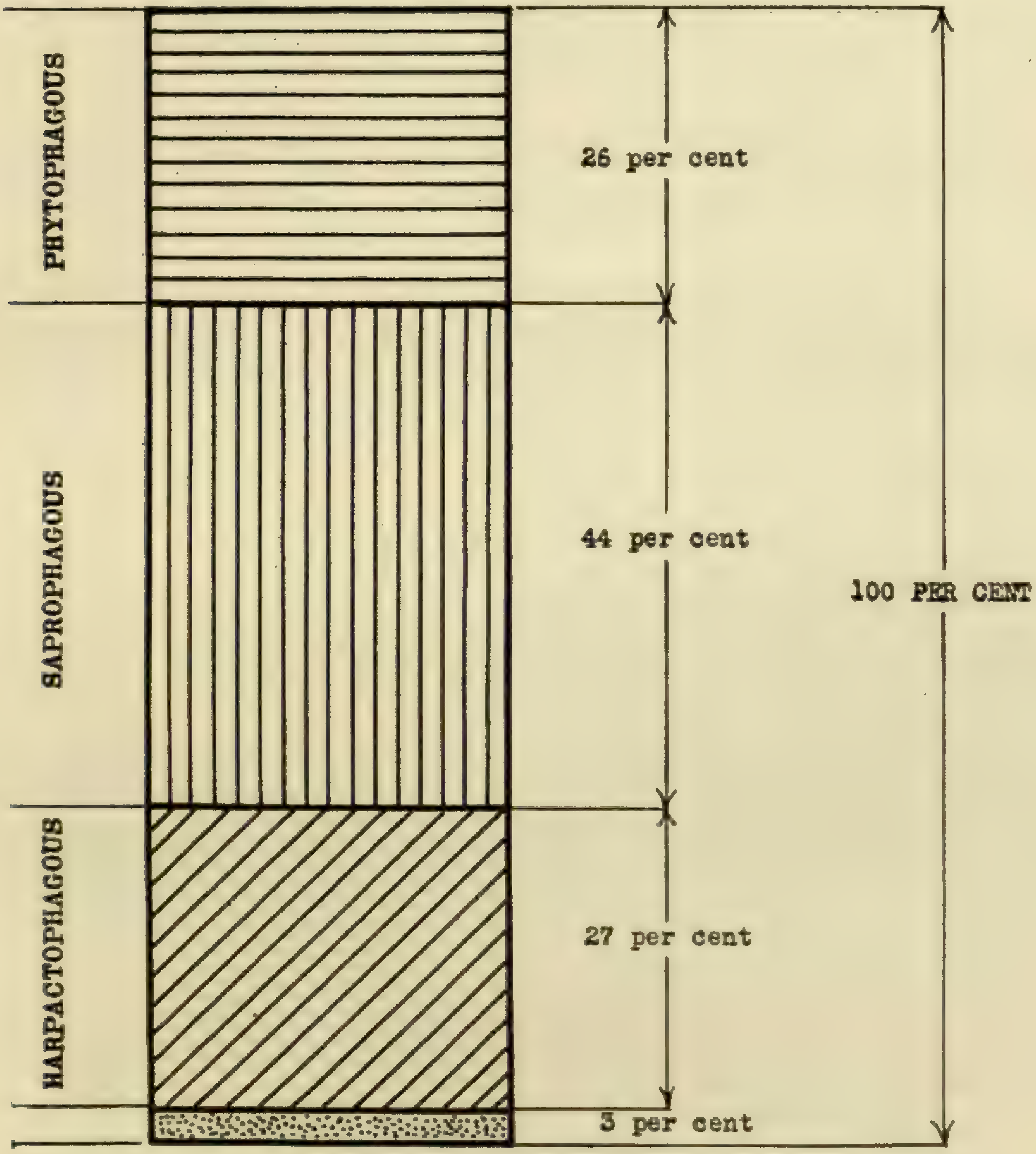

Diagram illustrating the comparative abundance of the various types of food-habits in the Coleoptera.

obscure have been grouped separately. In the Phytophaga have been placed those species which feed upon the higher plants. In the Saprophaga will be found those forms which feed for the most part upon disorganized tissue, vegetable and animal decay and such

species which remove or change the form of animal and vegetable remains and aid in reducing such substances into shape for assimilation by plants. While not strictly belonging to this group, species feeding on low forms of plants such as fungi and those living on dry vegetable and animal matter have been included for the sake of convenience and in order to avoid numerous subdivisions. In other words, the term Saprophaga is used in a very broad sense.

The Harpactophaga contains the predacious and carnivorous species, of which there are a great number, and whose activities help to preserve a natural balance between certain groups. Many of them are general feeders, appearing to be not particular whether their prey is a plant feeder or another predatory form. However, in some families, such as the *Coccinellidæ,* there is a decided specialization as to the prey, and such a group is very often an important specific check to unusual increases in the numbers of plant lice. The Coleoptera attacking living mammals are few in number. The species in the family *Platypsyllidæ* consists of a wingless beetle found on beavers. In the *Leptinidæ,* the species have been found in the nests of field mice and bumble-bees, but their exact habits are somewhat obscure. It has been suggested that the bumble-bee nest is the natural home and that the field mice afford transportation from one nest to another.

The last group is made up of those families of which little or nothing appears to be known concerning their food habits. While this same lack of information is true for a large number of individual species placed in the other groups, yet enough is known of their general family habits so that little risk is run in placing them as family units. This, however, could not be done with any certainty in the case of the last class and they are presented simply as a group difficult to classify from a food standpoint.

The following tables show the name of each family, the number of species in that family described up to and in-

cluding 1918 and a brief statement indicating the more important food habits. The information in the first two columns was compiled from the recently issued "Catalogue of the Coleoptera of America North of Mexico" by C. W. Leng.

PHYTOPHAGA

Family	No. Species	Habits
Lymexylidæ	2	Bore in hard wood.
Buprestidæ	379	Wood borers in healthy and unhealthy trees.
Cerambycidæ	1,123	Borers in dead, dying and healthy trees and plants.
Chrysomelidæ	974	Feeders on vegetable tissue.
Mylabridæ	93	In seeds.
Brentidæ	6	In wood.
Belidæ	1	Like *Curculionidæ*.
Curculionidæ	1,839	Feeders upon vegetable tissue.
Platypodidæ	5	Boring in trees.
Scolytidæ	379	Borers in wood, healthy and sick trees.

SAPROPHAGA

Family	No. Species	Habits
Silphidæ	137	Scavengers in dead animal and vegetable matter, in fungi.
Clambidæ	6	Same as above.
Orthoperidæ	57	In decaying vegetation, under bark, etc.
Staphylinidæ	2,748	Varied, in ants' nests, in fungi, in decaying animal and vegetable matter, etc., predatory.
Pselaphidæ	355	Varied, in ants' nests, under vegetable decay, in wet moss, in rotten stumps, etc.
Clavigeridæ	7	Same as above.
Ptiliidæ	83	In decaying vegetable matter, excrement, fungi.
Sphæriidæ	3	In vegetable decay.
Scaphidiidæ	50	In rotten wood, fungi.
Sphæritidæ	1	Same as *Silphidæ*.
Cupesidæ	4	Under bark, in dry wood, may be predaceous.
Œdemeridæ	49	In timber cast up by sea.
Mordellidæ	142	Varied, adults on flowers, larvæ in dead wood, fungi, stems of live plants.
Pythidæ	17	In timber.
Pyrochroidæ	11	Under bark of tree stumps.
Euglenidæ	39	In dead wood.
Cerophytidæ	2	Probably like those of *Elateridæ*.
Cebrionidæ	9	Probably like those of *Elateridæ*.
Elateridæ	576	In decaying wood, in soil on roots of grasses, etc.

Melasidæ	57	In dead trees.
Throscidæ	25	Like those of *Elateridæ*.
Dascillidæ	29	On roots of aquatics, in fungi.
Dermestidæ	129	In dried animal matter.
Ostomidæ	64	Varied, under bark, in granaries, in fungi, predaceous.
Nitidulidæ	132	Sap beetles, on flowers, predaceous.
Rhizophagidæ	14	Probably like above.
Monotomidæ	36	In ants' nests, probably have no relations with ants.
Erotylidæ	71	Mainly in fungi.
Cryptophagidæ	135	In fungi and decomposing vegetable matter.
Mycetophagidæ	32	Under bark, in fungi.
Colydiidæ	84	In fungus covered wood.
Lathridiidæ	104	In fungi.
Mycetæidæ	4	In fungi.
Endomychidæ	34	In fungi.
Phalacridæ	117	Under bark, on flowers.
Alleculidæ	124	Larvæ in rotten wood, adults on leaves, flowers.
Tenebrionidæ	1,139	In dry vegetable matter, fungi.
Lagriidæ	17	Probably like above.
Melandryidæ	81	In dry wood, fungi.
Ptinidæ	37	In dry animal and vegetable matter, wood, drugs, etc.
Anobiidæ	233	In dry vegetable matter.
Bostrichidæ	61	In dry wood.
Lyctidæ	16	In dry wood.
Sphindidæ	6	In fungi.
Cisidæ	85	In fungi.
Scarabæidæ	996	Varied, in decaying vegetation, on roots of plants, on green vegetation
Lucanidæ	30	In decaying wood.
Passalidæ	2	In decaying wood.
Platystomidæ	62	On dead wood, in fungi.

HARPACTOPHAGA

Family	No. Species	Habits
Cincindelidæ	114	Predaceous.
Carabidæ	2,165	Predaceous.
Omophronidæ	15	Predaceous.
Haliplidæ	41	Aquatic, predaceous.
Dytiscidæ	333	Aquatic, predaceous.
Gyrinidæ	41	Aquatic, predaceous.
Hydrophilidæ	190	Predaceous.
Scydmænidæ	174	Feeding on acari, in ants' nests.
Histeridæ	384	Found in same situations as scavengers but probably predaceous.

Lycidæ	50	Carnivorous as larvæ.
Lampyridæ	52	Larvæ carnivorous, adults on flowers.
Phengodidæ	23	Larvæ carnivorous, adults on flowers.
Cantharidæ	155	Larvæ carnivorous, adults on vegetation.
Melyridæ	321	Predaceous, adults on flowers.
Cleridæ	181	Predaceous, adults on flowers.
Corynetidæ	38	Predaceous.
Cephaloidæ	8	Probably similar to those of *Cantharidæ*.
Rhipiphoridæ	26	Larvæ parasitic in ants' nests, on cockroaches.
Meloidæ	227	Larvæ predacious, adults on green vegetation.
Cucujidæ	85	Varied, under bark, predacious, in stored products.
Coccinellidæ	362	Predaceous.

Animal Parasites

Family	No. Species	Habits
Platypsyllidæ	1	Animal parasite.
Leptinidæ	3	Probably parastic on mammals.

Food Habits Obscure

Family	No. Species	Habits
Amphizoidæ	2	Aquatic.
Brathinidæ	3	
Telegeusidæ	1	
Micromalthidæ	1	
Eurystethidæ	3	
Pedilidæ	54	Probably like those of *Anthicidæ*.
Anthicidæ	191	On surface of earth like ground beetles.
Plastoceridæ	19	
Rhipiceridæ	6	In wood.
Psephenidæ	4	Semi-aquatic.
Dryopidæ	17	Aquatic.
Helmidæ	36	Aquatic.
Heteroceridæ	11	Semi-aquatic.
Georysidæ	2	In wet places.
Helodidæ	32	Probably aquatic.
Chelonariidæ	1	
Byrrhidæ	97	Habits obscure, on ground beneath cover, about grass roots.
Rhysodidæ	4	
Derodontidæ	5	
Murmidiidæ	5	
Monœdidæ	1	
Monommidæ	6	

SUMMARY

	No. Species	Per Cent. of Tota
Phytophaga	4,801	26
Saprophaga	8,252	44
Harpactophaga	4,985	27
Animal parasites	4	
Food habits obscure	501	3
	18,543	100

About 26 per cent. of the species of Coleoptera are phytophagous, most of this percentage being made up of the families *Curculionidæ, Cerambycidæ* and *Chrysomelidæ.* Almost one half of the species of beetles, or 44 per cent., appears to be saprophagous for the most part and in this group the families *Staphylinidæ, Tenebrionidæ* and *Scarabæidæ* supply over half of the species. In the predaceous group, consisting of 27 per cent. of the total, the *Carabidæ* with its 2,165 species is the largest single contributor. Thus almost three fourths of the species of beetles in North America are apparently engaged in what we call useful activities.

INDIRECT EVIDENCE FROM DUPLEX HYBRIDS BEARING UPON THE NUMBER AND DISTRIBUTION OF GROWTH FACTORS IN THE CHROMOSOMES

DR. D. F. JONES

Connecticut Agricultural Experiment Station, New Haven

Sufficient evidence has accumulated to indicate that the main features of the chromosome theory of hereditary transmission, as worked out for *Drosophila,* are applicable to plants. Peas, primula and maize have been the best materials so far to demonstrate linkage of factors in plants. Owing to the ease of culture, large number of seeds produced and the great genetic variability the maize plant is becoming very useful in this line of investigation. The agricultural importance of the plant and the large number of people working with it have already made the list of Mendelian factors definitely determined large and increasing rapidly. Due mainly to the industry of Professor Emerson and his co-workers at Cornell University, six linked groups are already visible in rough outline, some of which have a goodly number of factors fairly well located. It therefore seems pertinent to consider some indirect evidence funished by this plant having a bearing upon the chromosome mechanism.

In working out the best means of utilizing inbred strains of corn for the purpose of increasing production it has been found to be advantageous to cross again two different first generation hybrids each of which were the result of combining two different self-fertilized families. Altogether four homozygous types, each differing from the other in many visible characters, are brought together in this way in a progeny which has an extremely complex composition. Assuming that the inbred strains have been reduced to complete homozygosity, the first generation hybrid is uniform. Statistical measurements show this

to be so. Theoretically, all the plants are hereditarily exactly alike. When such a hybrid with segregating gametes is again crossed with a similar first generation hybrid but having a different genetic construction, the result is a mixed lot of plants in which practically every individual differs in some degree germinally from every other.

This statement holds for any numbers that it would be possible to grow. Every inbred strain of maize, that has so far been obtained by continued self-fertilization with one progenitor in each generation, has differed in many ways from every other inbred line, whether they came originally from the same or different varieties. All the inbred strains coming from different individuals at the start show a noticeable increase in vigor when crossed and a rapid reduction of growth and great increase in variability in the immediately following generations when again self-fertilized. It is therefore not at all improbable that most of the self-fertilized strains differ from each other by a large number of genes in every chromosome.

If such is the case, then the duplex combination will have an extraordinary amount of genetic diversity. This may be made clearer in the following illustration. If, instead of being crossed, a hybrid was self-fertilized and there was only one factor difference in each pair of chromosomes, over one million plants would have to be grown in order to have an even chance of securing all the possible combinations (assuming maize to have 10 chromosomes). But with more than one factor in each chromosome the situation is far different. Two factors in each chromosome having a linkage ratio of 10 per cent. would necessitate 20^{20} individuals in the segregating generations to obtain the same result. This is calculated from the formula $[2(r+1)^{n-1}]^{2c}$ where $r+1$ is the linkage ratio, in this case 10 per cent., or $9+1$, n is the number of factors in each chromosome, and c is the number of chromosome pairs. This number of plants to be grown would require an area roughly 57,346 million times the total surface of the earth. But instead of being

self-fertilized, the hybrids with their segregating gametes are again crossed and certainly there are more than two factor differences in most of the chromosomes having varying degrees of linkage with each other. At present almost nothing is known about the heredity which the two first generation hybrids may have in common. But all the four homozygous types when crossed singly in the six possible combinations show about an equal amount of heterosis. The double-crossed combination shows no reduction in vigor of growth, but on the other hand this appreciably increased. This is due, however, in part to a better start as the plants come from large, well-nourished seeds grown on vigorous plants, whereas the first cross is handicapped in this respect.

The doubly hybrid plants are theoretically more diverse than self-fertilized second generation progenies coming from the same parents, but compared with the first and second generations the double cross has features of both. In respect to growth characters the plants are a group of many different first generation hybrids. Very little recombination can take place to allow recessive weaknesses to appear. In fact any recombination that does take place is probably out-balanced by an increase in heterozygosity in other factors. A critical comparison of such double hybrids with their parental first generation hybrids and with their second generation self-fertilized sibs in respect to variability of different characters ought to give some indication of the distribution in the chromosomes of the hereditary factors affecting growth.

In those factors which are independent of the growth of the plant the variability of the double cross should approach or exceed that of self-fertilized second generation. In those characters which are directly dependent upon the vigor of the plant the double cross should resemble more closely the first hybrid generation. Five characters have been taken and measured in three different but similar lots of plants. These are: number of rows of grain on the ear (pistillate inflorescence); nodes of plant, height of plant, length of ear; and pro-

duction of grain (weight of entire pistillate inflorescence with mature seeds). A previous study of a large number of first generation hybrids between inbred strains of maize has shown that the average number of rows of grain of the hybrids was increased 5.29 per cent. above the mean position of their parents; similarly nodes per plant 6.45 per cent.; height of plant 27.44 per cent.; length of ear 28.57; and total production of grain 180.00 per cent. The variability of these F_1 plants was slightly decreased below the parental average in nearly every case in respect to these characters.

Rows of grain and nodes are therefore much less influenced by the vigor of the plant than are the other characters, notably production of seeds, which is very largely determined by the amount and rapidity of growth. Assuming that the complementary action of dominant favorable growth factors is responsible for the vigorous growth of the hybrids, it would be expected that $F_1 \times F_1$ combination would not be as variable as the second generation resulting from self-fertilization in respect to production of grain per plant, provided a large number of essential growth factors were acting and that these were distributed rather uniformly throughout the chromosomes. On the other hand such characters as rows of grain on the ear and nodes per plant being largely independent of growth vigor, would not be expected to show a reduction in variability when compared with the second self-fertilized generation.

The distribution and statistical constants for the second generations grown from self-fertilized seed of the parental hybrids have been compared to the reciprocal crosses of the same parental hybrids in three different sets of plants. In each case the cross-fertilized seed, which produced the $F_1 \times F_1$ plants, and the self-fertilized seed, from which the F_2 plants were grown, came from the same ears. The two kinds of pollen were applied in a mixture at one time and the seeds separated by their color at maturity.

Without giving the extensive data upon which the

figures are based, the averages of the coefficients of variability of the $F_1 \times F_1$ and the F_2 families are brought together in table 1. With these are given some figures averaged from the F_1 parents. These are not from the exact first generation parents of the progenies used to

TABLE I

A COMPARISON IN VARIABILITY OF SINGLE FIRST GENERATION, DOUBLE FIRST GENERATION AND SECOND GENERATION HYBRIDS

Characters Measured	F_1		$F_1 \times F_1$		F_2	
	Ave. C.V.	Range P.E.	Ave. C.V.	Range P.E.	Ave. C.V.	Range P.E.
Rows of grain......	8.90	.40– .98	12.76	.52– .65	12.31	.45– .82
Nodes of plant.....	5.54	.25– .73	5.88	.25– .27	6.20	.26– .33
Height of plant....	7.06	.25–1.04	6.20	.25– .33	6.92	.26– ,40
Length of ear......	13.83	.72–1.66	13.23	.41– .75	16.90	.69–1.03
Weight of grain....	24.13	1.15–1.42	26.99	1.19–1.44	32.68	1.11–2.31

give the other results in Table I. They are similar but were not grown in the same years. They can not be compared as closely to the $F_1 \times F_1$ and F_2 lots as these can be compared with each other. The coefficients for variability of the $F_1 \times F_1$ and the F_2 plants, averaged from three different combinations with a fairly large number of plants in each grown from seed of which the two contrasted kinds came from the same ears, are strictly comparable. The greater growth of the double hybrids as shown by the increase of the means makes comparison of the coefficients of variability somewhat unreliable. The appearance of the plants in the field supports the statistical data, as it is the uniform production which makes the hybrid plants so valuable for agricultural purposes. There is a noticeable difference between the double cross and the self-fertilized second generation in even size, similar appearance and general excellence as the plants are harvested in the field.

The figures show that the variability of the $F_1 \times F_1$ families is about the same as the F_2 families in rows of grain and nodes per plant. In height of plant, length of ear and weight of grain per plant, all characters which

are markedly influenced by the vigor of the plants, there is a reduction in variability. Particularly is this true of length of ear and weight of grain, which are fairly reliable measures of the plant's reproductive ability, which in annual plants sums up the organism's entire energy. In other words the plants are uniformly vigorous and are not dependent upon exceptional individuals for their high average position. This is indirect evidence that those hereditary factors which are concerned with the growth of the plants are numerous and widely distributed throughout all or many of the chromosomes.

As a means of corn improvement it would be highly desirable to bring together into a pure breeding homozygous condition all those factors which cause the hybrid plants to excel their parents. Such individuals should be even more efficient in their growth processes than the heterozygous combinations of the same factors because the determiners responsible for hybrid vigor seldom show complete dominance. The recombination of linked factors is a problem that demands the most careful attention of the plant and animal breeder. It is the closely linked factors which are the main concern. When the distance between any two loci is fifty units or more, then all the factors situated outside of these points are independent of each other in transmission and it makes no difference from the standpoint of recombination whether the factors are in the same or different chromosomes. Therefore the number and arrangement of the individual genes themselves seem to be more important than the number of chromosomes. Although as yet it is impossible to compare the numbers of factors in different species, it does not seem likely that the genus *Rosa* with 8 chromosome pairs is genetically less complex than *Nicotiana* species with 24 pairs. Some crustacean species with 84 pairs of chromosomes are contrasted with various mammals with 8 to 12. Even in the Arthropods alone the haploid number ranges from 2 to 100. It seems profitless to look for any significance in chromosome, numbers. Leaving aside the matter of doubling of chromosomes any

differences that there may be are probably qualitative rather than quantitative. It is possible that there may be very little difference in the amount of essential hereditary material. But the word "amount" must be considered as equivalent portions. The visible size of the chromatin mass fluctuates greatly even at different stages of growth in the same individual.

Although the cytological proof of the chromosome theory is still so meager as to make speculation somewhat useless, nevertheless, looking at the matter from the standpoint of difficulty of recombination the important consideration is the number of fifty-unit lengths of chromosomes. However, the Morgan school unit of measurement, the one per cent. of crossing over, is not a stable unit, as they have shown that crossing over fluctuates rather disconcertingly, due both to environmental and germinal modifying factors. Detlefsen[1] finds the rate of crossing over between certain loci to be very profoundly altered by continued selection for high and low cross over stock. So that for the present the terms proposed by Haldane[2] of *morgan* and *centimorgan* as measures of chromosome length do not have any precise application. At the same time the rate of crossing over is the only measure available and can not be given up until a better one is found. The term *morgan,* referring to a one-hundred-unit length of chromosome is convenient but does not have the biological significance that a fifty-unit length of chromosome would have. Since every gene is independent in transmission from all other loci in the same chromosome more than fifty units distance from it, and has the usual Mendelian relation with them as well as with all the factors in the other chromosomes, the term *mendel* would perhaps be useful, if the employment of such terms can be justified at all. Applied in this way a *mendel* is a measure of chromosome length equivalent to fifty per cent. of crossing over.

It should be noted that a *mendel* is not comparable to a

[1] *Proceedings of the National Academy of Science,* 6: 663–670, 1920.
[2] *Journal of Genetics,* 8: 299–309, 1919.

single short chromosome fifty units long. It is not to be thought of as a fixed portion of any chromosome. The chromosomes are, or course, not to be considered as marked off into fifty-unit lengths. But the result of recombination with a large number of factors is approximately the same as if such were the case. Because it brings out the fact which has not always been fully appreciated, that recombination within a chromosome takes place as easily as between different chromosomes, when the distance between the loci is sufficiently great, the term *mendel* as a measure of chromosome length may have some value.

In the primitive unicellular organisms it is conceivable that the hereditary substances were not located in a mechanisn as well regulated as in the higher organisms. As specialization increased, the grouping of factors in chromosomes has undoubtedly been of very great evolutionary significance. The chromosome mechanism has been subjected to natural selection as severely as any external morphological feature and has developed coordinately with sexual reproduction—the one to make recombination possible, the other to make that process orderly.

Although it is largely speculation it seems necessary to believe that there is some functional relation between the factors associated together in a chromosome or portion of a chromosome. There is some evidence for this in the quick and exact return of certain species hybrids to one or the other parental type. Evidently only those individuals resulting from gametes in which crossing over has not occurred are able to live. So far the factors which have been located seem to be placed at random in the chromosomes, and it is impossible to make out any significant relation among them. This in itself may be an indication of an immense number of hereditary determiners which play a part in the organism. For as yet the function of only the relatively superficial factors can be seen. The vitally important ones can not be dispensed with and therefore can not be studied except as the lethal factors show some effect in hybrid combination.

EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

II. Hereditary Differences in Duration of Life in Line-bred Strains of Drosophila [1]

PROFESSOR RAYMOND PEARL AND SYLVIA L. PARKER

Introduction

It was shown in the first paper in this series (27)[2] that there was a marked difference in mean duration of life, and in the form of the l_x curve, between wild-type stocks of *Drosophila* on the one hand and the synthetic quintuple mutation stock on the other hand. It was further made clear that, because of the technique used in the experimental work, there could be no doubt that the basis of this difference must be hereditary and not environmental. Furthermore, Hyde (11) and Pearl (6) have presented evidence for the Mendelian inheritance of this character duration of life.

Given it to be the fact, as the just cited work demonstrates to be the case, that there are hereditary differences within the same species of *Drosophila* in respect of duration of life, the problem which next presents itself is to determine whether *within* a particular strain of *Drosophila* hereditary differences exist, and if so what their magnitude may be, their degree of permanence, etc. In

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, The Johns Hopkins University, No. 48.

[2] A word of explanation is necessary as to the method of handling bibliographic references in this series of papers. In the first paper a list of 26 references numbered consecutively from 1 was appended. It is proposed not to duplicate references in any subsequent paper in the same series. Consequently the first *new* bibliographic citation in the present paper is numbered 27. When any reference is made to titles already cited in the first paper in the series, the numbers which they bear in the list appended to that paper will be used. This practice will be adhered to in all subsequent papers in this series of Studies.

short one wishes immediately to get a kind of knowledge for this organism and character similar to that which Johannsen (28, 29) got for the size character of beans from his pure-line work. The first, and in a sense preliminary, investigations on this problem will be presented in this paper. Later in the series we expect to publish much more extended and penetrating evidence on the same problem. Some, however, must be presented early in the series in order to make the account of subsequent experiments intelligible.

It is obvious that in the case of an organism like *Drosophila* it is impossible to have a pure-line in the strict sense of Johannsen. The most that one can do is to have inbred lines, and the most intense degree of inbreeding possible in the premises is by brother × sister mating. The general plan of the experiments reported in this paper can be outlined as follows:

1. Mate a virgin brother and sister, chosen at random each from the same one of the original 5 foundation stocks (cf. 27).

2. Repeat this for as many pairs as the facilities of the laboratory make possible.

3. Test the progeny of each mated pair separately for duration of life, and form for each group of such progeny a life table.

4. Each such mated pair constitutes the beginning of a line, in which at any time the processes noted under paragraphs 1, 2, and 3 above could be repeated. In this paper will be reported the results of one such repetition.

The general technique of the experimental work has been fully described in the first paper of this series and need not be repeated. It should merely be emphasized again that the environmental conditions in respect of food, housing, temperature (25° C.) and atmospheric conditions were identical for all the flies in the experiments here reported.

Duration of Life in Different Progeny Groups out of Brother × Sister Matings

The survivorship data (l_x frequencies) for 7 progeny groups each out of a mating of brother × sister are exhibited in Table II. All distributions are put on the same basis of 1,000 flies at emergence from the pupal stage. The absolute numbers of flies involved in each experiment are given at the foot of each column. These numbers are

TABLE I

Brother × Sister Matings. First Test

Lines	Original Stock (Described in (27))	Date of Mating	Date of Emergence
100.....	Old Falmouth	April 8, 1920	April 19–May 3
101.....	" "	April 7, 1920	April 17–May 2
201.....	New Falmouth	April 7, 1920	April 18–May 2
202.....	" "	April 10, 1920	April 20–April 29
300.....	Sepia	April 7, 1920	April 17–May 3
301.....	"	April 6, 1920	April 17–May 3
303.....	"	April 8, 1920	April 18–May 2

TABLE II

Survivorship Distributions of Progeny of Brother × Sister Matings. Both Sexes Together

Age in Days	Numbers of Survivors up to Indicated Age in Lines No.						
	100	101	201	202	300	301	303
1	1,000	1,000	1,000	1,000	1,000	1,000	1,000
6	983	993	1,000	689	926	870	882
12	937	987	1,000	607	858	727	764
18	891	934	952	492	809	602	702
24	811	901	943	426	623	441	621
30	743	875	857	344	549	342	522
36	589	855	790	197	383	255	429
42	514	770	600	148	272	205	311
48	406	599	505	148	148	130	261
54	240	493	381	49	105	75	168
60	91	296	219	33	12	31	112
66	29	99	133	16	6	12	56
72	6	20	10	0	0	2	0
78	0	7	10	—	—	0	—
84	—	7	0	—	—	—	—
90	—	0	—	—	—	—	—
Abs. No. of flies	175	152	105	61	162	161	161

smaller than is desirable, but these experiments represent a relatively early stage of the work before the technique of getting maximum progenies for life table work had been perfected. Further it must be remembered that the individuals in any column are the progeny of only one single pair of parents. The source of the lines together with other pertinent data are shown in Table I.

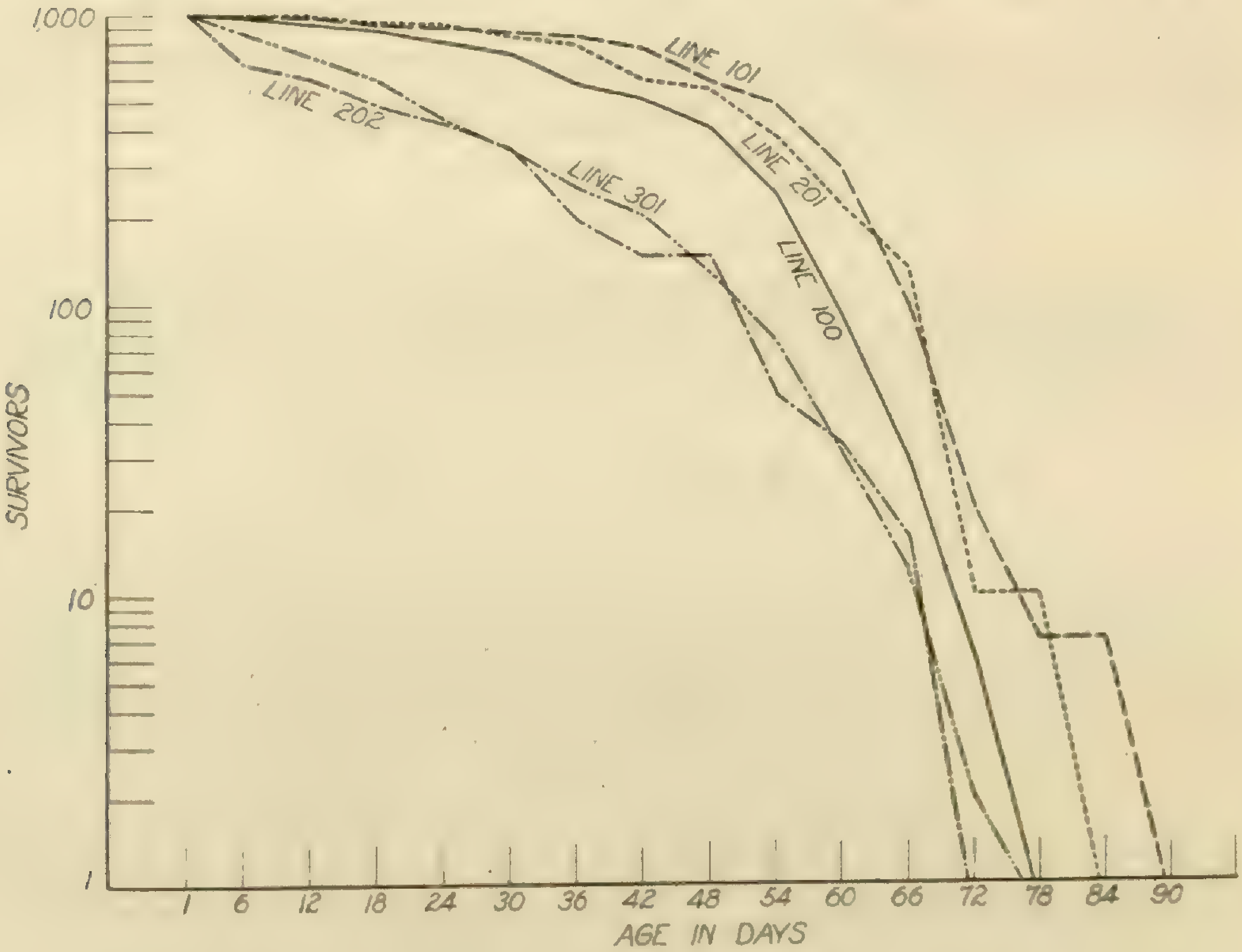

FIG. 1. Survivorship (l_x) graphs for lines 100, 101, 201, 202 and 301.

Five of these distributions are shown graphically in Fig. 1, and their biometric constants are given in Table

TABLE III

FREQUENCY CONSTANTS FOR d_x DISTRIBUTIONS. FIRST TEST

Line No.	Mean Duration of Life (Days)	Standard Deviation (Days)	Coefficient of Variation
100.....	40.45 ± .84	16.38 ± .59	40.49 ± 1.68
101.....	50.02 ± .85	15.51 ± .60	31.01 ± 1.31
201.....	47.40 ± .99	15.03 ± .70	31.71 ± 1.51
202.....	22.04 ± 1.57	18.18 ± 1.11	82.49 ± 7.74
300.....	31.19 ± .83	15.76 ± .59	50.53 ± 2.33
301.....	25.28 ± .92	17.25 ± .65	68.24 ± 3.56
303.....	32.02 ± 1.07	20.04 ± .75	62.59 ± 3.14

III. In calculating these constants, the absolute d_x frequencies, and not the per mille frequencies, were of course used.

From these data it is at once apparent that these progeny groups show distinct, and in some cases decidedly large, differences both in mean duration of life and in the form of the mortality distributions. Lines 101 (Old Falmouth stock) and 201 (New Falmouth stock) show the longest mean duration of life, and they are sensibly identical in the form of the life curve, having regard to the errors of random sampling. The difference in the means for these two lines is 2.62 ± 1.31 days, an obviously insignificant difference, only 2 times its probable error. Similarly these two lines do not significantly differ in absolute or relative variability, the difference between the standard deviation being $.48 \pm .92$.

Line 100 (Old Falmouth stock) has a distinctly and significantly lower mean duration of life than 101 or 201. Comparing it with line 101 the difference in the means is 9.57 ± 1.20 days or approximately 8 times its probable error. The l_x curve lies throughout its course below the lines for 101 and 201. Line 100 is also relatively more variable in duration of life than 101 and 201, but largely because of the difference in the means.

The individuals in line 202 (New Falmouth stock) are the shortest lived of any here dealt with, and the shortest-lived wild-type strain we have as yet isolated. Its mean duration of life is less than half that shown by lines 101 and 201 and only a little more than half that of line 100. Line 202 shows the highest relative variability in duration of life of any of the lines here discussed. It also has the highest absolute variability with one exception (line 303).

Lines 300, 301 and 303 (Sepia stock) are all relatively short-lived lines. 300 and 303 are substantially identical, while 301 has a lower mean approaching that of line 202. These sepia lines are also characterized by high relative variability.

Results of Inbred Re-tests for Constancy

During the progress of the experiments described in the preceding section the offspring flies (from original brother × sister matings) in each of the lines, whose duration of life was being tested, were allowed to mate at random in their bottles, and their progeny removed to form stocks of the several lines. These stocks were allowed to reproduce in stock bottles, all matings being therefore random *within the line,* for a period of about 7 months (cf. Table IV). At the end of that time it was decided to make a re-test of each line to see how it was then behaving relative to duration of life. There was then made, at dates indicated in Table IV, a random selection from each line stock bottle from which a brother and sister pair was bred, and these two individuals were mated to get a set of progeny on which to carry out a second set of life duration experiments. The necessary facts as to line numbers and dates on this re-test are given in Table IV.

TABLE IV

Brother × Sister Matings. Second Test

Line from which Second Selection of Brother and Sister Was Made	Number of Line of Progeny of Second Brother × Sister Mating	Date of Original Brother × Sister Mating	Date of Second Brother × Sister Mating
100	104	April 8, 1920	November 6, 1920
101	107	April 7, 1920	October 14, 1920
201	207	April 7, 1920	October 18, 1920
202	208	April 10, 1920	October 14, 1920
300	304	April 7, 1920	November 6, 1920
301	307	April 6, 1920	October 14, 1920
303	309	April 6, 1920	October 14, 1920

The survivorship distributions of the progeny groups of this second brother × sister mating are given in Table V, and the biometric constants calculated from the observed d_x distributions in Table VI. These tables are for comparison with Tables II and III above.

TABLE V

Survivorship Distributions of Progeny of Second Brother × Sister Matings. Both Sexes Together

Age in Days	Numbers of Survivors up to Indicated Age in Lines No.						
	104	107	207	208	304	307	309
1	1,000	1,000	1,000	1,000	1,000	1,000	1,000
6	997	1,000	973	833	1,000	862	1,000
12	923	950	926	738	870	700	978
18	871	926	819	643	870	623	911
24	713	917	792	595	674	469	700
30	629	901	785	500	478	392	489
36	552	860	711	286	435	285	456
42	469	777	644	167	261	177	267
48	395	686	530	0	152	92	89
54	304	595	430	—	109	46	67
60	178	488	255	—	0	23	44
66	66	264	141	—	—	8	0
72	0	83	20	—	—	8	—
78	—	8	20	—	—	8	—
84	—	0	7	—	—	0	—
90	—	—	0	—	—	—	—
Abs. No. of flies	286	121	149	42	46	130	90

TABLE VI

Frequency Constants for d_x Distributions. Second Inbred Test

Line No.	Mean Duration of Life (Days)	Standard Deviation (Days)	Coefficient of Variation
104	39.59 ± .74	18.63 ± .53	47.06 ± 1.62
107	53.74 ± 1.07	17.40 ± .75	32.38 ± 1.54
207	45.34 ± 1.10	19.97 ± .78	44.04 ± 2.03
208	25.65 ± 1.53	14.68 ± 1.08	57.23 ± 5.42
304	32.09 ± 1.43	14.43 ± 1.01	44.97 ± 3.75
307	25.22 ± .99	16.70 ± .70	66.22 ± 3.79
309	33.00 ± .91	12.84 ± .65	38.91 ± 2.23

The purpose of this second test was, of course, to see to what extent duration of life was holding constant in the line. During the period between the first and second test the stocks of the several lines had been subjected to varying environmental influences, in particular in relation to temperature, the stock bottles having been kept at room temperature, which varied rather extensively. Did the lines after 7 months have the same characteristic life curves that they exhibited on the first test? Allowing 12

days from generation to generation in the case of flies reproducing freely at random in stock bottles, the interval elapsing between the first and second tests would cover roughly almost 18 generations. This is a long period and affords abundant opportunity for change in the average genetic constitution of the population.

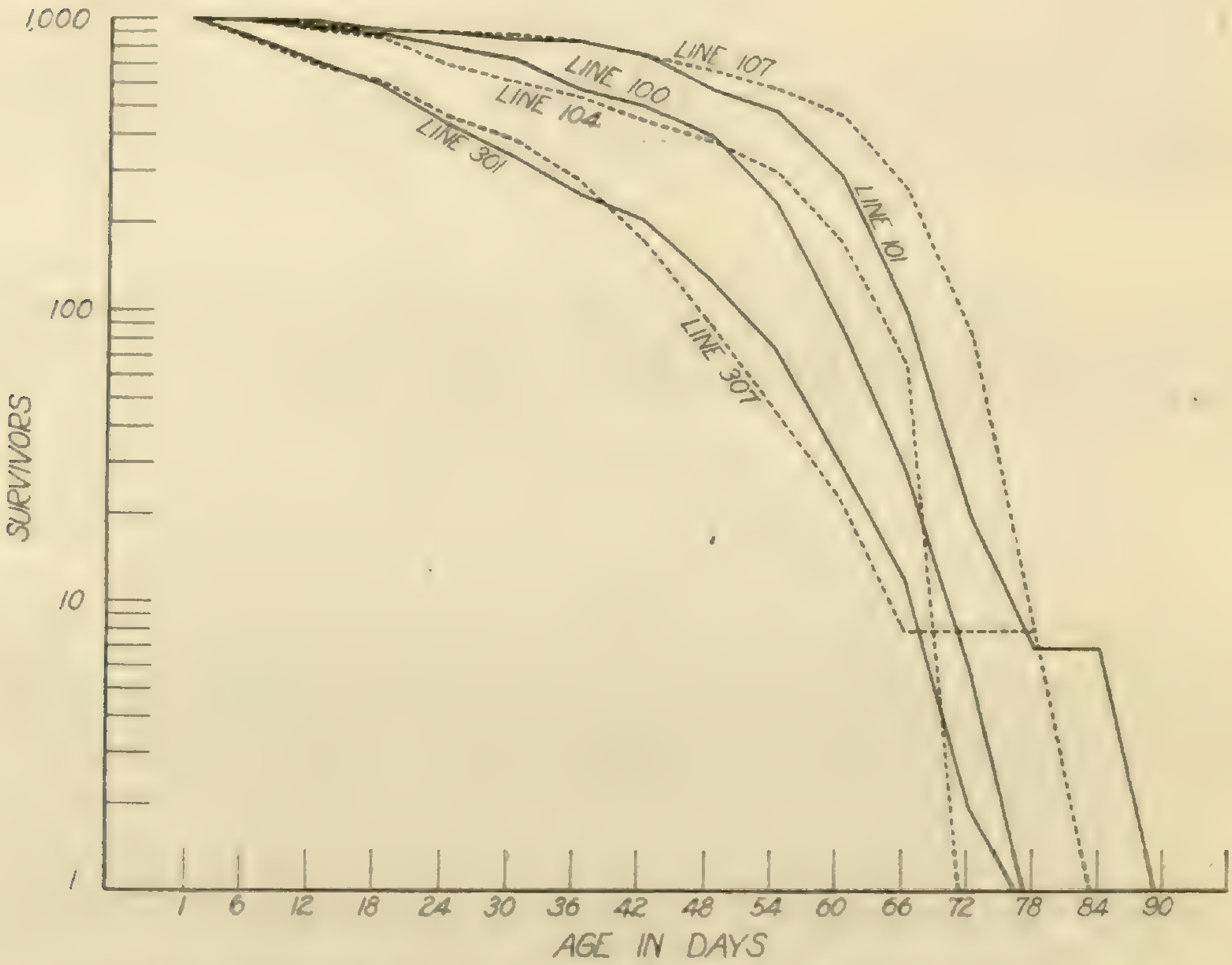

FIG. 2. Comparing the l_x lines of the first and second inbred tests of lines 101, 100 and 301.

An examination of Tables V and VI and Fig. 2 shows at once, in a general way, that the characteristic features of the several lines in respect of duration of life did in fact hold remarkably constant during this period. A more precise comparison of the means is made in Table VII.

There can be no question of the substantial constancy of these lines, over the period covered in the tests in respect of duration of life. The l_x curves run well together till the upper end of life is reached, where, because of the small numbers involved, there is some irregularity. In no case is the difference between two comparable means, as shown in Table VII, as much even as three times its prob-

able error, nor is there any certainly significant change in variability having regard to the probable errors of the differences involved.

TABLE VII

Differences in Mean Duration of Life Between the First and Second Inbred Tests of the Several Lines

Corresponding Lines (Mean of Second Test Minus Mean of First)	Difference of Means (Days)	$\frac{\text{Diff.}}{\text{P. E. Diff.}}$
104–100	− .86 ± 1.12	.77
107–101	+ 3.72 ± 1.37	2.72
207–201	− 2.06 ± 1.48	1.39
208–202	+ 3.61 ± 2.19	1.65
304–300	+ .90 ± 1.65	.54
307–301	+ .06 ± 1.35	.04
309–303	+ .98 ± 1.40	.70

Results of Mass Culture Re-tests for Constancy

The point may well be made that in the re-tests of the lines described in the preceding section an additional element is introduced in the fact that the flies for the re-test were the progeny of a second brother × sister mating. What one wishes to know is: what degree of constancy in duration of life is exhibited by the general stocks in each line, mating purely at random, after the initial selection and inbreeding? We wish now to present some data on this point. Table VIII gives the biometric constants for this material. Mass culture re-tests have been made on two of the original lines, 100 and 101. These mass culture re-tests were made in two ways as follows:

(*a*) From the stock bottles of the line to be tested a large sample of progeny was taken at random each day as the flies emerged from the pupal stage, and these progeny flies were put in small bottles for a duration of life experiment in the usual way described in (27).

(*b*) From the stock bottles of a particular line to be tested a number of virgin flies (usually 8 to 10 of each sex) were taken at random immediately upon emergence, and mated as a group in a mating bottle. The progeny from this sample was then removed, upon emergence, to small bottles and a regular duration of life test carried as described in (27).

TABLE VIII

FREQUENCY CONSTANTS FOR MASS CULTURE RE-TESTS. ORIGINAL LINES

Line No.	Test	Dates of Emergence	Numbers of Flies	Mean (Days)	Standard Deviation (Days)	Coefficient of Variation
100	Mass Culture	1920 Sept. 25–Oct. 21	433	33.05 ± .76	23.48 ± .54	71.05 ± 2.31
	Original brother × sister	1920 Apr. 19–May 3	175	40.45 ± .84	16.38 ± .59	40.49 ± 1.68
Difference		5 mo. 12 days	—	− 7.40 ± 1.13	+ 7.10 ± .80	+30.56 ± 2.68
101	Mass Culture *A*	1920 Sept. 25–Oct. 21	473	53.09 ± .70	22.53 ± .49	42.44 ± 1.09
	Mass Culture *B*	1921 Mar. 18–Apr. 4	124	48.53 ± 1.02	16.76 ± .72	34.54 ± 1.65
	Original brother × sister	1920 Apr. 17–May 2	152	50.02 ± .85	15.51 ± .60	31.01 ± 1.31
Difference *OA*		5 mo. 13 days	—	+ 3.07 ± 1.10	+ 7.02 ± .77	+11.43 ± 1.70
Difference *OB*		10 mo. 29 days	—	− 1.49 ± 1.33	+ 1.25 ± .94	+ 3.53 ± 2.11

It is at once apparent that the mass re-tests on line 101 gave extremely satisfactory results as to constancy of duration of life in the line, after intervals of approximately 5 and 11 months. The mean value for either the *A* or the *B* test does not significantly differ, having regard to its probable error, from the mean shown on the original test at the start of the line. The mean of the *A* mass re-test almost exactly agrees with that of the second inbred test of the same line, as given in Table VI.

In the case of line 100, the mass re-test after 5½ months approximately does not give such close agreement. The mean is significantly lower, the difference being 6.6 times its probable error. No explanation of this result is, as yet, forthcoming, but it probably means no more than lack of genetic purity in the line. It is, however, interesting to note that the sense of the change is in the same direction as that in which line 100 in general differs from line 101, which we regard as our most typical wild-type line in respect of duration of life. That is, line 100 is, as compared with 101, a shorter-lived line. Its mass culture re-test is still shorter lived.

The variability in respect of duration of life, whether measured in absolute or relative terms, is uniformly higher and in two cases out of the three by a significant amount in the mass culture than in the original inbred tests. This is, of course, exactly what would be expected on general genetic grounds. One brother × sister mating, as has been shown by Pearl (30), Jennings (31) and others, reduces the heterozygosis in the strain by only 50 per cent. It is interesting to note, in connection with the explanation suggested above for the difference in the means in the case of line 100, that the variability in the mass re-test on that line is very much higher than in the original inbred test.

A mass re-test was carried out on two of the lines from the second brother × sister matings. The results from these experiments are presented in Table IX.

TABLE IX

FREQUENCY CONSTANTS FOR MASS CULTURE RE-TESTS. TWICE INBRED LINE

Line No.	Test	Date of Emergence	Numbers of Flies	Mean (Days)	Standard Deviation (Days)	Coefficient of Variation
107	Mass Culture *A*	1921 Apr. 19–Apr. 22	1,338	49.74 ± .25	13.69 ± .18	27.52 ± .54
107	Second brother × sister *S*	1920 Oct. 25–Nov. 4	121	53.74 ± 1.07	17.40 ± .75	32.38 ± 1.54
101	Original brother × sister *O*	1920 Apr. 17–May 2	152	50.02 ± .85	15.51 ± .60	31.01 ± 1.31
Difference *SA*		6 mos. 9 days	—	− 4.00 ± 1.10	− 3.71 ± .77	− 4.86 ± 1.63
Difference *OA*		11 mos. 27 days	—	− .28 ± .88	− 1.82 ± .96	− 3.49 ± 1.42
309	Mass Culture *A*	1921 May 18–25	468	34.04 ± .34	11.02 ± .24	32.38 ± .79
309	Second brother × sister *S*	1920 Oct. 25–Nov. 1	90	33.00 ± .91	12.84 ± .65	38.91 ± 2.23
303	Original brother × sister	1920 Apr. 18–May 2	161	32.02 ± 1.07	20.04 ± .75	62.59 ± 3.14
Difference *SA*		6 mos. 28 days	—	+ 1.04 ± .97	− 1.82 ± .69	− 6.53 ± 2.37
Difference *OA*		13 mos. 1 day	—	+ 2.02 ± 1.12	− 9.02 ± .79	−30.21 ± 3.24

The substantial constancy of line 101, in both mass and inbred tests, is evident. In respect of variability the line behaved somewhat like 303 discussed below.

In line 303 again the constancy of the line in respect of mean duration of life is as definite as could be expected. Over periods of approximately 7 and 13 months, the mean duration of life has not sensibly changed, having regard to the probable error involved. The results respecting variability are somewhat anomalous. Both the second inbred and the mass re-test show variability of a distinctly lower order than was exhibited by the progeny of the original brother × sister mating. It seems probable that the original test by accident gave a variability result higher than was really characteristic of the line. But the mass culture re-test exhibits a lower variability, not certainly significant, to be sure, than the first test on line 309. Of course it is to be expected that with continued brother × sister mating the variability of mass cultures from the line would come nearer and nearer to that of a further inbred lot of progeny from the same line. Probably the results of Table IX are an expression of the realization of such expectation, obscured by the fact that the numbers are small and the errors of sampling consequently relatively large.

Discussion and Summary

The data presented in this paper appear to demonstrate, with comprehensiveness and accuracy, three broad facts.

A. That there exist in a general population of *Drosophila melanogaster* (or its mutants) genetic differences in respect of duration of life.

B. That these genetic differences are capable of isolation, by appropriate selection and inbreeding.

C. That within an even moderately inbred line, the genetic differences in duration of life remain constant over periods of at least 10 to 25 or more generations.

These facts, based upon the determination experimentally of the duration of life of 3,039 individual flies in 18 experiments, under constant environmental conditions, place this character "duration of life" in the category of genetically definite and workable characters, and indicate that it will just as well repay careful analytical study as the characters more usually dealt with. Furthermore, duration of life is a character of great general biological significance.

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SHORTER ARTICLES AND DISCUSSIONS

NOTE ON A CASE OF HUMAN INBREEDING[1]

THROUGH the kindness of a friend the following pedigree is presented. It is that of a family of English stock, which has been in this country since the early eighteenth century, and during that time has been one of the principal families of a rural community.

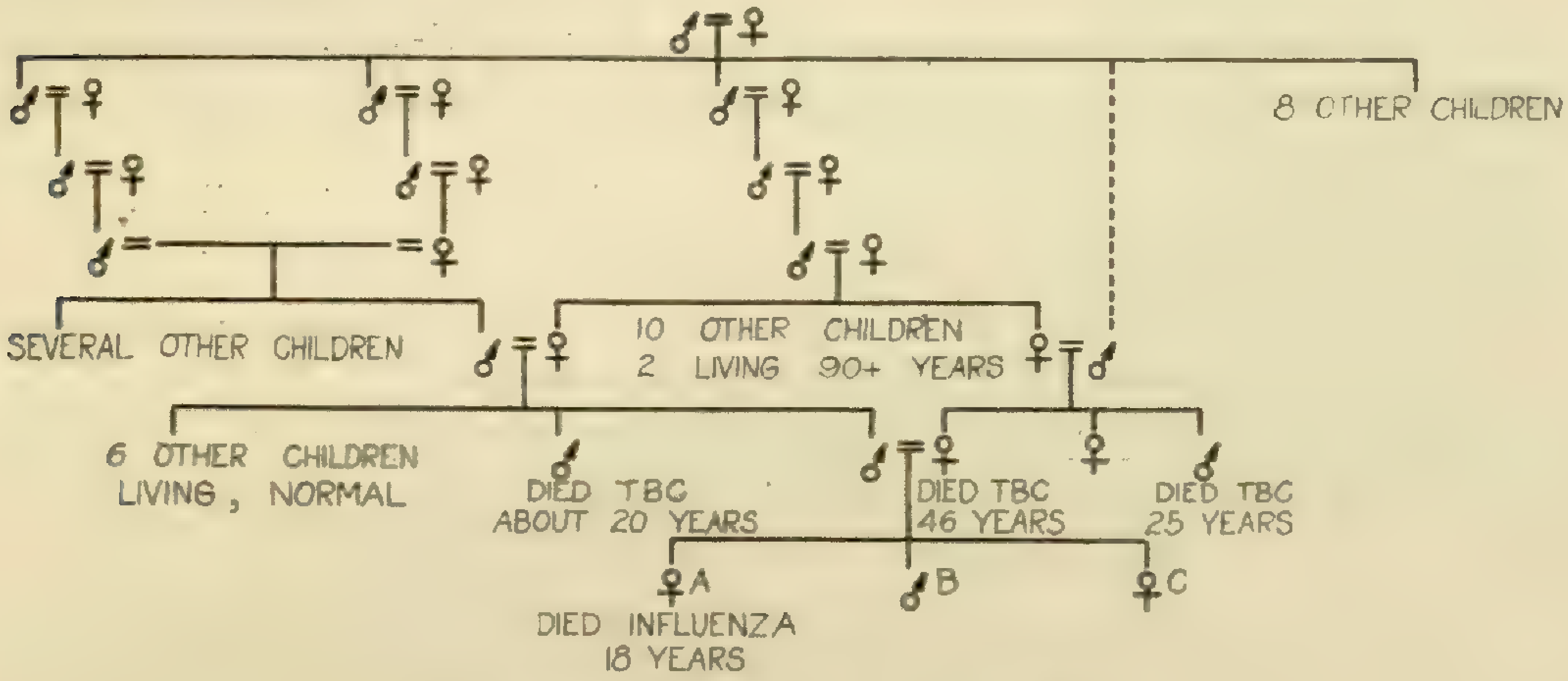

FIG. 1. **Pedigree of an inbred family.**

To quote from my correspondent's letter, "A was a fine young girl. She had graduated from high school but did not go to college as her mother had died in the summer and she wished to take charge of the home. B is about 16 years old. A splendid young man, bright and apparently healthy. He is in school standing about average. C is 10 years old or thereabouts. An exceptionally bright child and one that is very much alive and full of spirit."

Assuming that the line of descent represented in the figure by a broken line, indicating that the number of generations is not known, includes the same number of generations as the other lines the coefficients of inbreeding[2] for the children in the last

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University. No. 41.

[2] Pearl, "Studies on Inbreeding," I–VIII, AMERICAN NATURALIST, 1913–17.

generation (viz., A, B, and C) are as follows:

$$Z_1 = 0, \qquad Z_4 = 25,$$
$$Z_2 = 25, \qquad Z_5 = 34.37,$$
$$Z_3 = 25, \qquad Z_{T_5} = 27.1,$$

i.e., in five generations of ancestry the inbreeding is about a quarter of the possible maximum.

There is no deleterious effect of inbreeding apparent in this pedigree. The three children in the last generation, the most inbred of any, show no signs of abnormality. In their father's fraternity, for which $Z_1 = Z_2 = Z_3 = 0$; $Z_4 = 12.5$; $Z_{T_4} = 4.1$, or in four generations of ancestry there is $1/25$ of the possible maximum inbreeding, one of eight died of tuberculosis; the other seven have attained adult age. In the mother's fraternity, for which $Z_1 = Z_2 = Z_3 = 0$; $Z_4 = 6.25$; $Z_{T_4} = 2.0$, two out of the three have died of tuberculosis. The least inbred, therefore, show the greatest susceptibility to tuberculosis. The numbers are, of course, too small to draw any certain inference, but so far as they go, they accord best with the view that there is no harmful effect of inbreeding per se.

JOHN RICE MINER

ON COLOR VARIATIONS IN CHITONS

THE question was raised by Bateson ("Materials," 1894, p. 307) as to whether variation occurring in serial parts whose repetition is not strictly speaking of a metameric sort, would be found to simultaneously affect each of the parts involved in such a series. With this point in mind he examined a collection of chitons, the 8 shell plates of these animals providing an excellent opportunity for such observations. He found color variations affecting all the plates of an individual to be of rather rare occurrence, but that plates 2, 4 and 7 seemed, on the other hand, to exhibit a decided tendency to vary together (in several species of *Chiton*).

Although the problem of metamerism, so far as it concerns variation, has perhaps lost some of its original attractiveness, I have thought it worth while to point out that in *Chætopleura* several curious types of shell variation are apparent, involving either simultaneous variation throughout the series or variation in a single shell-valve alone, or both.

The commonest type of color pattern, in *Chætopleura apiculata*, is one which involves a double band of blackish or grayish pigment running the length of valves 2 to 8, the bands joined on 2 and on 8 by continuous semicircular blotches (cf. Fig. 1, *d*).

In 3 out of 219 specimens, however, a central dark stripe, clearly marked on valves 2 to 8 inclusive, accompanied a much fainter double band extending to valve 1 (Fig. 1, *b*), and made up of regular triangular grayish blotches on the posterior borders of valves 2 to 7. In one case, a bright central band of white

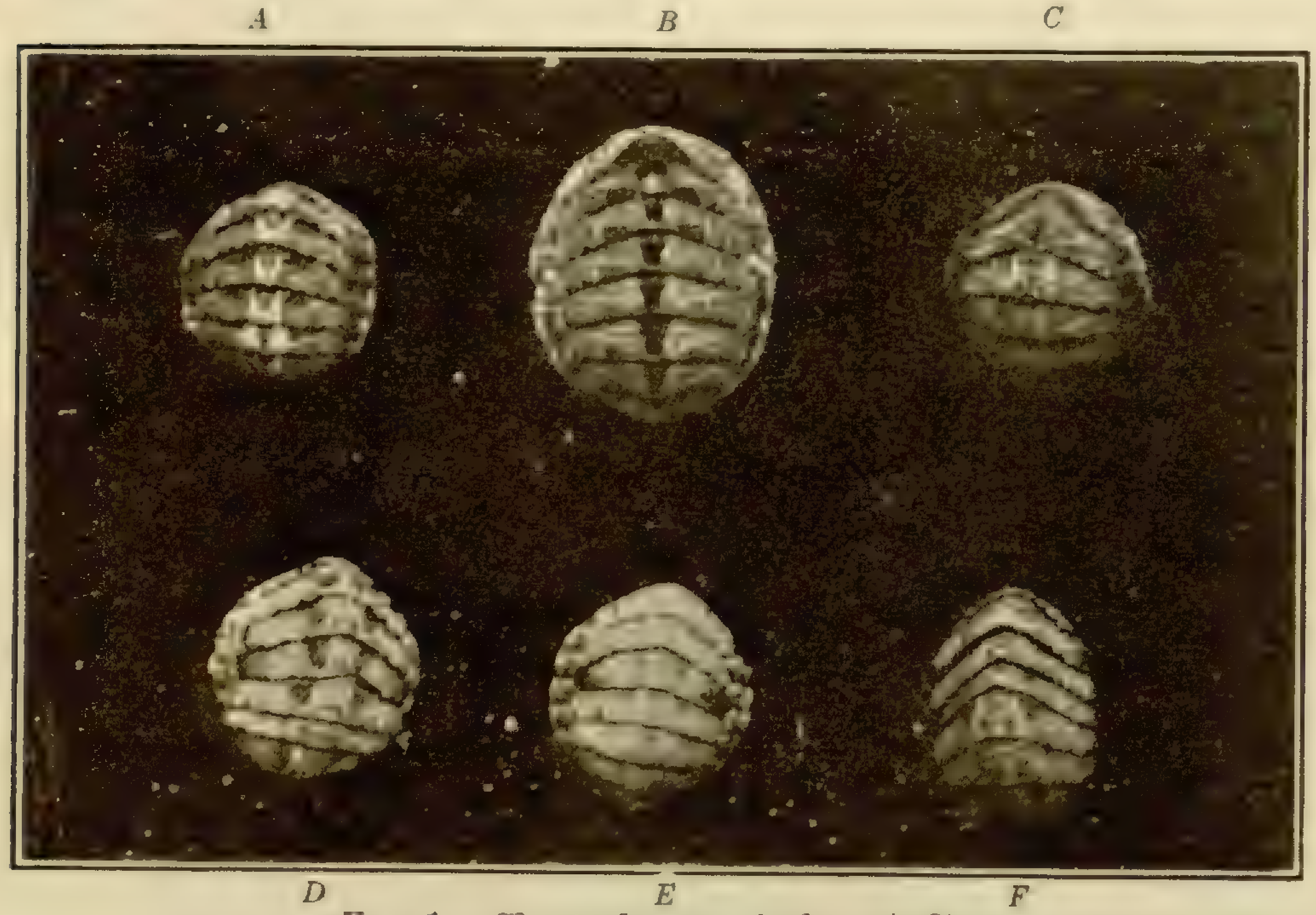

FIG. 1. *Chaetopleura apiculata* (×2).

was marked by a median grayish blotch on valves 3 to 7 (Fig. 1, *a*); a paler example of the same kind is shown in Fig. 1, *e*.

These are instances where variation from the more usual color type clearly has taken place simultaneously in the whole series of valve-plates, this condition being seen not only in the form of the central stripe, but also in the shapes of the individual pigment blotches comprised in the double band (cf. Fig. 1, *a* and *b*), as well as in a few cases where three small but distinct pigment flecks were noted on the lateral field of each plate.

In addition, however, two sorts of pattern variation occur which are quite different from the foregoing. In three instances a definite yellow or orange central blotch appeared on valve 2, and nowhere else (Fig. 1, *e*). And in five further instances there was found a marked blackish blotch at either lateral margin of valve

4 (Fig. 1, *d, e*). In two further cases, this type of lateral marking was continued in the form of less distinct marginal blotches on valve 3. The marginal blotches on valve 4 may accompany an otherwise "normal" pigment pattern (10 examples in 219 examined; Fig. 1, *d*), or may be present where there is evidence of a tendency for the formation of a distinct axial stripe (five examples; Fig. 1, *e*).

It is evident, then, that in Chitons color pattern variations may occur in such a way as to affect single valves only (and, in *Chætopleura,* specifically valve 2 or 4); and either quite independently of this type of variation or accompanying it, may also affect all valves in the series simultaneously. Such variations are quite independent of age.

W. J. Crozier

Rutgers College

FUGITIVE NET-VEINING IN THE CICADA (HEMIPTERA)

Tillyard has lately noted[1] that, besides the chitinized veins which serve for the support of the insect wing, there exist in some cases at least fugitive blood-veins during the expansion of the wing, which later collapse and more or less completely disappear when the wing dries. In the Lepidoptera 1st A and the base of M are veins of the same character, and possess tracheæ like other longitudinal veins in that order. In every particular except the absence of chitinization these appear to be true veins, and in forms where the veins are provided with special series of setæ, as in Acræa, they are often similarly supplied.

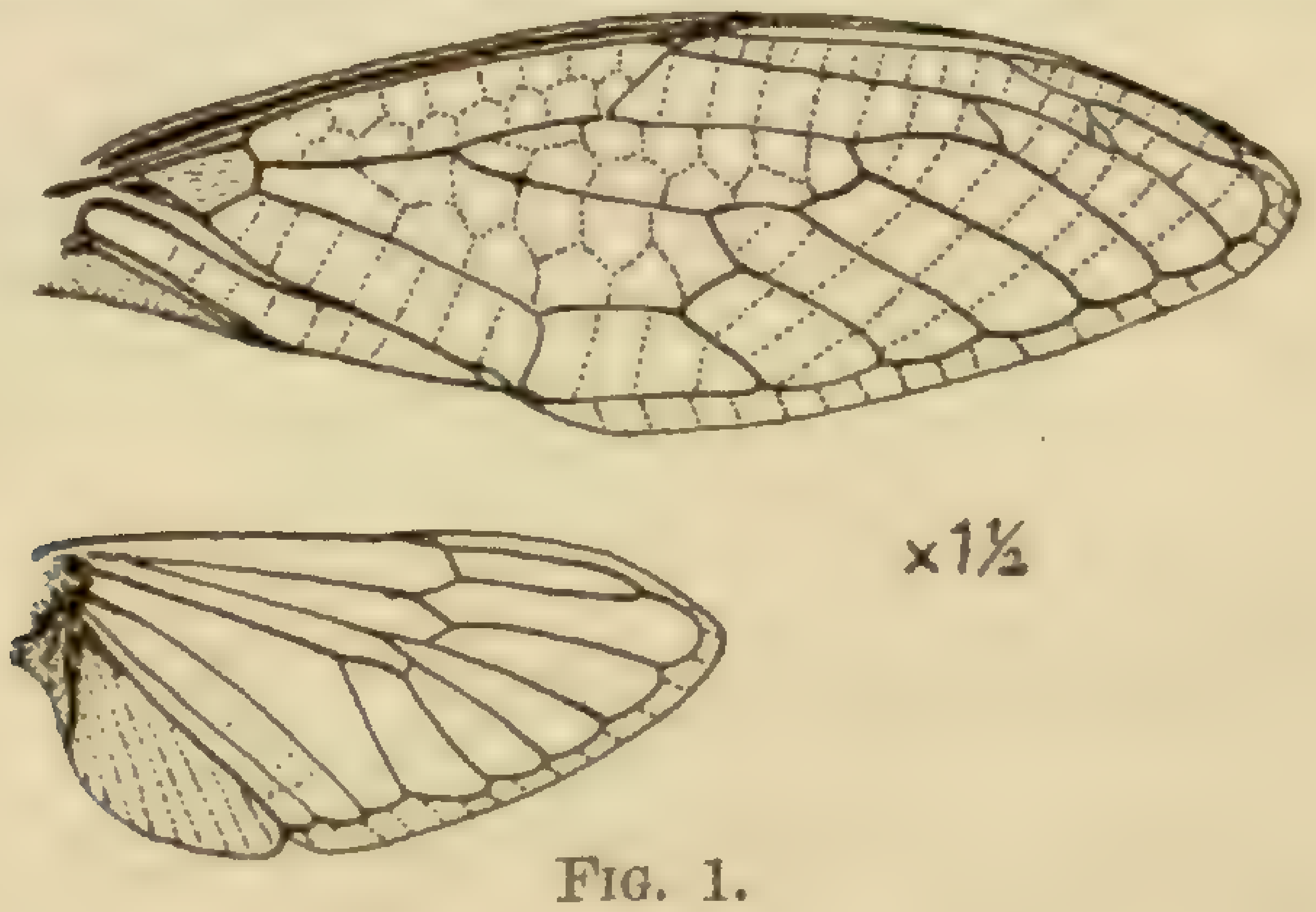

Fig. 1.

In watching a cicada expand, recently, I saw appear, as the

[1] *Proc. Linn. Soc.,* New S. Wales, 44, 621; 1919.

expansion approached completion, a regular system of blood-veins in the spaces between the permanent veins. These show plainly only in the few minutes when the wing has become partially transparent, but in the adult wing they produce a characteristic waviness of the membrane, and a few of them may be seen in a favorable light as faint white lines. The arrangement is perfectly definite: the narrow cells are filled by a series of simple, evenly spaced, cross-veins, while in cells R, 1st M_2 and M they form a double series of cells alternating with each other. On the narrow margin beyond the ambient vein they are evenly spaced, the regular longitudinal veins each ending opposite the middle of a marginal cell. Toward the costa there are two veins opposite each definitive cell, while opposite cells M_3 and M_4 there are three, and opposite cell Cu_1 there appear to be four. The margin of the hind wing is similar, but the disc of the wing was not observed. In the large triangular anal cell (3d A_1), instead of cross-veins there is a series of closely spaced parallel longitudinal veins, which remain visible in the dried wing.

It seems possible that these structures are the relic of a net-veining such as occurs in the Neuroptera. The different arrangement in the anal region is especially suggestive, as it would correspond to the plaited portion of the wing in the Orthoptera, where there exist numerous parallel longitudinal veins.

The figure is drawn from memory so far as the fugitive veins are concerned, checked up by the few that could be traced in the dry wing; it can be trusted only approximately.

Wm. T. M. Forbes

Cornell University,
Ithaca, New York

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IS THERE A TRANSFORMATION OF SEX IN FROGS?

PROFESSOR W. W. SWINGLE

OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY

THIS paper is a reply to the recent article of Dr. Emil Witschi which appeared in a late issue of the NATURALIST (Vol. LV, No. 641). Witschi is quite convinced that the problem of sex development and differentiation in frogs has been settled, and that nothing further remains to be said. However, the writer feels that instead of being solved, the time has come for a revision of the entire question of sex development in Anurans, and that the subject is ripe for a reinterpretation upon a more rational basis than that accorded to it heretofore.

The first portion of the paper will be devoted to a brief exposition of the writer's interpretation of sex in frog larvæ based upon data obtained from a study of the bullfrog. The second part of the paper is a reply to certain questions raised by Dr. Witschi.

In larval males of the bullfrog two gonads are formed, just as there are two kidneys formed, a pro-testis or embryonic sex gland destined to degenerate and disappear in ontogenetic development and a definite or functional testis which replaces it. The germinal elements of the pro-testis arise in the entoderm and migrate into the germ ridges early in embryonic life. The cells multiply rapidly and together with the mesodermal elements of the germ glands form paired ridges projecting into the cœlomic cavity. While the tadpole is very immature and has yet a year of larval life before metamorphosing, the

germ cells of the pro-testis undergo a precocious and abortive sexual cycle culminating in degeneration and resorption. Beautiful cysts of spermatocytes are formed, but the first maturation division rarely proceeds past the

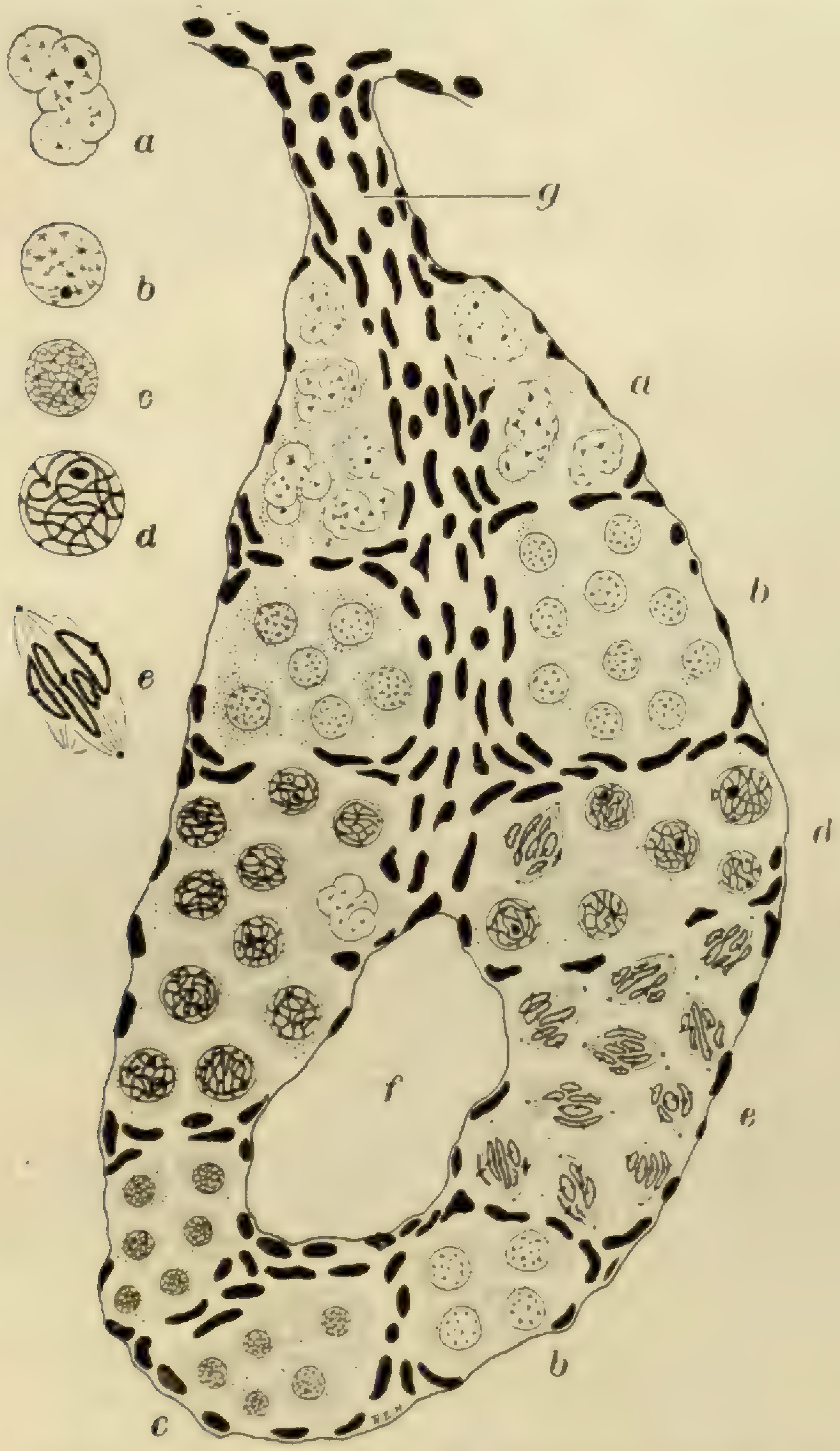


FIG. 1. Transverse section pro-testis *R. catesbeiana* tadpole. Animal has a year of larval life remaining. *A*, Spermatogonia showing nuclear polymorphism due to incomplete fusion chromosomal vesicles; *B*, Final spermatogonia; *C*, Spermatocytes in leptotene stage; *D*, Amphitene and pachytene; *E*, Heterotypic mitosis; *F*, Secondary genital cavity; *G*, Anlage of sex cords of definitive gonad which develops as a core within pro-testis.

anaphase owing to fragmentation of the centrosome and consequent formation of polyasters (Fig. 1). Sometimes aberrant spermatids are formed by suppression of the first and second maturation divisions and growth of axial filaments from the centrosome. Practically all the germ cells of the pro-testis degenerate and disappear while in various stages of maturation—some undergo an oviform type of degeneration, *i.e.*, hypertrophy enormously and take on the superficial characters of oocytes. The oviform type of degeneration, however, is more characteristic of the short larval-lived frogs than of *R. catesbeiana*, for in many animals these large cells appear rarely and in others not at all and this is an important point to keep in mind. This type of degeneration will be discussed in detail in a later paper; suffice to say it gives no clue to the sex of a cell. (See Plates 1 and 2.)

Some cells of the pro-testis fail to take part in the abortive sexual cycle persisting through the phase of maturation and degenerate as spermatogonia. These elements migrate into the sex cords (Fig. 1, *g*) which have formed meantime, and form a core of germinal tissue extending through the center of the pro-testis. This core of tissue plus the sex cords is the anlage of the definitive testis and is quite distinct from the pro-testis, the cells of which are maturating and degenerating, whereas the cells of the forming functional gonad remain as primitive spermatogonia. The definitive testis by rapid growth completely supplants the pro-testis which usually disappears some time before metamorphosis. The functional gonad is generally fully formed at metamorphosis when the larvæ are two years of age. Some tadpoles, but not all, develop ripe spermatozoa in the gonad at metamorphosis due to a second sexual cycle of the germ cells of the definitive gonad. (Swingle, '21, *Jour. Exp. Zool.*, Vol. 32.)

In the frogs with short larval-life the same succession of gonads occurs, but in these forms the developmental processes are greatly accelerated and the pro-testis ma-

turation cycle is cut short by the cells early becoming senescent and undergoing oviform degeneration *i.e.,* hypertrophy to such an extent as to superficially resemble oocytes. This oviform degeneration occurs to an even more marked degree in the progonad of the toad which has a still shorter larval-life, *e.g.,* in Bidder's organ. In male anurans the entire pro-testis or larval gonad is the homologue of the male organ of Bidder in *Bufo.*

The pro-testis of the short larval-lived frogs has been misinterpreted as an ovary owing to the oviform-type of degeneration characteristic of many of its senescent cells, and hence tadpoles are said to develop first as females, fifty per cent. later transforming into males. The normal embryological process by which the definitive testis develops as a central axis through the degenerating pro-testis or larval Bidder's organ, has been described by Witschi as the transformation of female tadpoles into males. In *R. catesbeiana,* where the larval life is prolonged over two years, the true nature of the pro-testis is revealed, for relatively few of the cells are of the oviform type and all transition stages between such cells and normal spermatocytes occur. The evidence presented by this material will be published in due time, and is too clean-cut to admit of any doubt that the entire larval gonad of male anurans is simply an embryonic male sex gland rudiment and not a temporary ovary. Witschi's Fig. 6 (this journal, Vol. LV), which he supposes is an ovary transforming into a testis is simply a transition stage in the development of the definitive testis, and degeneration of the pro-testis or Bidder's organ in a short larval-lived frog. Compare his Fig. 6 with Fig. 1 of this paper and note how the true male character of the cells of the pro-testis comes out in *Rana catesbeiana* tadpoles.

When the facts are considered it is evident that the transitory gonadic rudiment of male frog larvæ is an organ of Bidder which degenerates and is replaced by the definitive gonad. Any one who has studied the oviform-like

cells of the so-called sexually intermediate tadpoles and compared them with the cells of Bidder's organ in male toads, is at once struck by the remarkable similarity in their origin, development, structure and fate in the two groups. They are identical. The crux of the problem is the nature of Bidder's organ in male Bufonidæ and of the oviform-like cells of the pro-testis. The advocates of sex transformation have assumed that such cells are undoubtedly female, but no proof has ever been advanced that they are. Their ultimate fate is the same as that of the first year spermatocytes in the bullfrog tadpole—degeneration (see Plates 1 and 2). The sex-transformationists have been misled by the idea that everything superficially resembling an oocyte is necessarily such, or that any cell in tadpoles and first-year animals undergoing the early growth stages, leptotene, pachytene, etc., is to be regarded as female. These are fallacious criteria. Enormously hypertrophied oocyte-like cells which have passed through the early growth stages and entered the "germinal vesicle" period so characteristic of oocytes, occur as normal features of the male sexual cycle of certain animals, *e.g.*, myriapods (Figs. 5–8). These animals were at first regarded as hermaphrodites by Blackman (1905, *Bull. Mus. Comp. Zool.*, Harvard, Vol. XLVIII, no. 1) who found upon examination, however, that these "oocytes" were in reality spermatocytes of giant proportions, and developed into spermatozoa. The writer has examined some of Professor Blackman's material and the oocyte-like character of the male sex-cells is remarkable. In the material examined these cells practically fill the gonads. Firket, 1920, working on the chick embryo, describes and figures spermatocytes undergoing oviform degeneration, *i.e.*, enlarging to such an extent as to resemble oocytes. There are many other cases reported in the literature. How does Witschi know that the transitory oocyte-like cells he describes in the future male tadpoles or so-called hermaphrodites, are female cells and not senescent organ of Bidder cells occurring

in the course of the abortive and degenerate sexual cycle of an embryonic pro-testis?

The work of Witschi on the problem of sex in anurans can be summarized thus: He has described in great detail and with admirable exactness the process of development of the pro-testis or Bidder's organ in the short larval-lived frogs, its degeneration and final replacement by the definitive gonad. This process he calls transformation of females into males. The experimental investigations of Witschi upon sex transformation by environmental influences consists of this: By means of such agents as heat or cold, etc., he has simply modified the normal course of development of the pro-testis—Bidder's organ, thereby accelerating or delaying the development of the definitive testis. The experimental results show that it is possible to modify the developmental rate of the embryonic testis. Similar experiments carried out with regard to other larval structures would unquestionably give similar developmental modification. Cold hinders metamorphosis and all the normal structural changes metamorphosis implies. All of these environmental influences are interferences with the normal cycle of the gonads, by which the development of the definitive gonad out of the pro-testis is accelerated, retarded, or possibly prevented entirely. The following quotation from Witschi '14, page 10, is significant in this connection:

> Bei seinen Untersuchungen war es Hertwig aufgefallen, das unter dem Einfluss verschiedener Aussenbedingungen sich nicht nur die Geschlechtsziffern, sondern oft auch in ganz auffälliger Weise der Rhythmus, in Welchem die Keimdrüsen und manche andere Organe sich anlegen und entwickeln.

It is probable, judging from certain experiments reported, that the degree of development attained by the larval gonadic rudiment, its position in relation to the definitive gonads, its period of persistence, non-formation in some forms, and such like, may vary in different frog species and is determined by heritable factors. For example, in *Bufo*, the structure persists throughout life in

males, disappears after two years in females, and is anterior to the functional gonads. In frogs it forms the outer husk of the germ gland enclosing the centrally developing functional testis and may or may not show the oviform type of degeneration, *e.g.*, *R. catesbeiana.*

If sex is so labile in tadpoles and young frogs, and females so readily transform into males under environmental stimuli, why is it that such sex reversals do not occur in adult frogs *after the degeneration of the protestis* and the formation of the definitive testis has occurred? All investigators are agreed that the sex ratio of adult frogs of all species reported is approximately 50 – 50. If environment (ever changing in the same locality, and never the same in different regions), plays such an important sex transforming rôle, why do male tadpoles never transform into females — all investigators agree that they do not. Why do only fifty per cent. of the so-called larval females transform into males if they were not zygotic males from the beginning, and why do not all female frog larvæ transform into males instead of only fifty per cent. if such transformation is possible? Appeal cannot be made to Professor Hertwig's well-known late fertilization experiments because in these experiments the influence of the over-ripeness of the egg upon the zygotic conditions determining sex are unknown. Hormones! To date there is no positive evidence that such secretions have ever actually changed a female germ cell into a functional male germ-cell.

Cases of hermaphroditism in adult frogs are thought by some to furnish evidence of a sex transformation in frogs. However, true hermaphroditism in adult frogs is as rare a phenomenon as it is in mammals when we consider the few recorded cases, and the enormous number of frogs annually dissected the world over. Crew ('21), *Journal of Genetics,* Vol. II, no. 2, has summarized the recorded cases of abnormal sexual organs in frogs and states that there are forty cases. To this number should be added a recent case described in the bullfrog, making

forty-one. Among these forty-one cases, there are but twenty-seven true hermaphrodites. Crew's cases, twenty-one to thirty-three, inclusive, are not hermaphrodites, nor is case thirty-eight, as none of the animals possess ovotestes and some are entirely without gonads. True hermaphroditism in frogs is a permanent and pathological condition, probably due to a mix-up in the genetic constitution of the individual, and is not to be confused with the present problem which has to do with a normal but transitory embryological process.

Much has been written about the marked "sex potencies" of various portions of the gonads in so-called sexually intermediate frogs, *i.e.*, females transforming into males. It is claimed that the outer rind of the gonad exerts a profound female sex influence, while the inner portion exerts a purely male influence. Germ-cells remaining in the outer husk (the main portion of the larval gonad by the way) of the gland are female, those migrating into the central part among the sex cords become male. All such speculations are based upon misinterpretations. The outer portion or husk of the larval male gonad is simply the pro-testis, the cells of which are undergoing a precocious maturation cycle just as they do in the organ of Bidder in *Bufo*, the inner portion or sex cord region is where the definitive gonad begins development and as it spreads and grows the embryonic male gonad degenerates and disappears. *It is in the region of most marked "female" tendencies that the writer finds in the bullfrog entire cysts of unmistakable spermatocytes, and occasional spermatids* (Fig. 1, *e*). In other words, the pro-testis—what Witschi regards as an ovary—can in the bullfrog, where its development is greatly prolonged, give rise to practically mature male sex products. Recently, the writer made an observation of considerable interest. In the degenerating Bidder's organ (pro-testis) of a two-year-old male larva in which formation of the definitive testis had been delayed until metamorphosis and in which the oviform type of degeneration

was the most marked of any animal yet observed, several cysts of unmistakable spermatocytes and spermatids were observed. They arose from the maturating cells of what Witschi regards as the female part of the gonad — in reality the pro-testis, and were of the cell type characteristic of the adult frog. This observation shows two things clearly: (1) *That the direct descendants of the male primordial germ cells* (*pro-testis elements*) *can produce practically mature germ cells;* (2) *that the spermatocytes of the structure regarded by the writer as a pro-testis are really male cells, and that the structure in so-called sexually intermediate frogs and tadpoles is in no sense to be regarded as female in character.*

Another point is of interest here — the writer has never observed direct testicular development in *R. catesbeiana,* though it probably occurs in some strains; the indirect method alone has been found, *e.g.,* first a protestis is formed which is later supplanted by the definitive gonad. In the bullfrog, which has the longest larval life of any anuran, the pro-testis persists longer than in other forms, sometimes two years before giving place to the definitive gonad. What the writer calls a pro-testis of so-called sexually intermediate tadpoles is according to Witschi a transitory ovary. If this is true why is it that despite its persistence for such a long time, relatively few oocyte-like cells are found in *R. catesbeiana* and in many individuals none, throughout a two-year period, but instead the structure produces spermatocytes and sometimes spermatids? Why is it, if this structure is an ovary in the so-called females that later transform into males, that the shorter the larval life of male anurans, the more the pro-testis in its structure and behavior resembles the Bidder's organ characteristic of male toads, due to rapid oviform degeneration of its cells; the longer the larval life, *e.g., Rana catesbeiana,* the more the germinal elements undergo a normal sexual cycle characteristic of male cells? The answer is, because in forms with extraordinary prolonged larval lives the

true nature of the embryonic male gonad has sufficient time to manifest itself before being supplanted by the definitive gland.

We come now to a discussion of the nature of Bidder's organ in *Bufo*, for this is the classical example of oviform degeneration of racially senescent germ cells. Heretofore, this embryonic sex gland rudiment has been regarded as characteristic of toads, but such is not the case. In frogs the pro-testis or larval gonad is a Bidder's organ, destined to be replaced by the definitive male gonad developing within; in male toad larvæ on the other hand, the functional gonad arises behind the pro-testis or Bidder's organ, consequently this structure persists as a degenerate gonadic rudiment attached to the functional gland.

According to the writer's view, Bidder's organ in *Bufo* is simply a vestigial larval gonad persisting throughout life and has the same sex as the definitive gonad behind it—male in males, female in females. It is just as though the pro-nephros of tadpoles persisted as a non-functional and degenerate rudiment at the end of the mesonephros. That many such larval and embryonic rudiments do persist through adult life in various animals is a commonplace of embryology, and their persistence in one species and total disappearance in another related one, is also well known. Bidder's organ in *Bufo* then, is a persisting, in frogs a transitory, embryonic sex gland rudiment, a relic of a phylogenetically earlier sexual condition. The functional gonads are more recently acquired structures (like the larval mesonephros) superimposed upon the older degenerate glands. Briefly stated, the evidence for the view that Bidder's organ is homologous to the pro-testis of frogs and that it is not a rudimentary ovary except in female animals is as follows:

1. The cells of Bidder's organ in *Bufo* are unquestionably germ cells. The gland appears very early in embryonic life (two weeks after hatching) and its cells far

outstrip in development the cells of the definitive gonads located posteriorly.

2. The cells of Bidder's organ extremely early in development undergo a precocious and abortive maturation cycle and become senescent and degenerate oocyte-like structures when the germinal elements of the functional gonads have barely started to multiply to form the definitive glands. This occurs in individuals of both sexes.

3. The larval maturation cycle such as occurs in the bullfrog, and in other anurans, throughout the entire larval gonad is confined to Bidder's organ in *Bufo,* and the changes occurring in this structure do not affect the normal developmental cycle of the definitive germ glands behind.

4. The so-called transformation of female animals into males, claimed by Witschi and others to be the normal course of development in frogs, does not occur in toads. Why? Because in *Bufo,* the definitive gonads are from the beginning located posterior to Bidder's organ, and it is not necessary in order that they may develop that this structure degenerate and disappear as is the case in frogs where the definitive testis starts development as a core within the pro-testis or Bidder's organ, necessitating its complete destruction.

5. Few have ever claimed that sex in toads is labile and easily reversed by environmental influences. Why? Because the sex of the definitive gonads is definitely fixed and clear cut at an early stage of life. The separation of Bidder's organ and the gonads has precluded the possibility of confusing the pro-gonad and the definitive gonad.

6. Bidder's organ is merely a persisting embryonic gonad whose cells have undergone oviform degeneration. It is not a rudimentary ovary except in female animals. This is indicated by its presence in both sexes in toads; its presence in Spengel's case of true hermaphroditism; by the fact that neither in male or female of toads do

its cells develop into true functional eggs; and by its degenerate structure from its inception in both sexes.

In a recent paper (*Zoologischer Anzeiger,* Dec., 1921) Harms describes marked hypertrophy of Bidder's organ following testis removal. He considers that castration of males causes Bidder's organ to develop into an ovary. However, it should be noted that such operated animals with hypertrophied Bidder's organ (ovary according to Harms) retain all their male secondary sex characters, and their normal mating instincts and that these male characters and instincts undergo a normal cyclical development in such induced "females." When Harms removed both testes and Bidder's organ the somatic sex characters and instincts failed to develop, showing clearly that Bidder's organ in male toads acts like a testis in maintaining the secondary sexual characters. This is excellent evidence for the writer's view that in male toads Bidder's organ is simply a persisting embryonic male sex gland rudiment and not an ovary. If it is an ovary why should it develop and maintain the secondary sex characters of the male in absence of the testis?

7. Recent investigators have inclined to the view that this structure is a hermaphrodite gland, *i.e.,* in male toads a rudimentary ovary, in females a rudimentary testis. If this is true then the admission is made that large, senescent, oocyte-like germ cells are not necessarily female cells—the crucial point for which the writer is contending.

8. Bidder's organ in *Bufo* corresponds to the larval gonad of frogs which in these forms disappears in the male and is replaced by the definitive testis. In the case of female anurans so far as the writer is aware no one has carried out a thorough investigation of the germ cycle from larval to fully adult life to see whether or not such a degeneration occurs in the female line. In mammals and birds such degeneration of the female embryonic line of germ-cells is quite well established as the work of Winiwarter, Firket and others shows.

The writer is of the opinion that it is only by adopting the view advanced here regarding the homologous nature of the larval male gonad of frogs, and Bidder's organ in *Bufo*, that the problem of sex differentiation in anurans can be placed upon a rational basis. The theory accords with the embryological facts, covers the experimental finding of Witschi and others, accords with our own cytological data in the bullfrog, accords with the embryonic sexual conditions of other vertebrates, *i.e.*, the degeneration of the embryonic line of germ cells in birds and mammals, and lastly furnishes an explanation of Bidder's organ in *Bufo*.

The key to the puzzle of sex development in frogs is simply this: every cell that superficially resembles an oocyte is not necessarily a female cell especially when occurring in an otherwise male individual, and that the larval male gonad of anurans is an organ of Bidder — a rudimentary embryonic sex gland with the same sex as the definitive gonad arising out of it. Misinterpretation of oviform hypertrophy and degeneration of racially senescent sex cells has rendered chaotic the problem of sex differentiation in anurans (see Plates 1 and 2).

Witschi regards the development of certain somatic sex characters such as the Müllerian ducts as very positive evidence for his theory of sex transformation. He says:

> In males which show a typical development of the testicles, no Müllerian ducts of any significance are formed. On the other hand, such animals as first develop ovaries and later undergo the transformation of sex, also show regular oviducts; and these continue to grow just up to the time when the transformation of sex begins. This parallelism in the behavior of the Müllerian ducts and the gonads furnishes definite proof that the "eggs" and "ovocytes," described by the writer, are in fact really eggs and ovocytes and that the transformation of sex is a well-established fact. After the transformation of sex, when the ovocytes have disappeared, the Müllerian ducts begin to shrink but they do not disappear completely, etc., etc.

The following data shows that in reality such so-called parallelism in the behavior of the Müllerian ducts and

the gonads does not exist and that evidence based on such parallelism is worthless.

In the normal males of adult *Rana pipiens* the Müllerian ducts are remarkable for their size and degree of development. They arise as cellular cords in the peritoneum at the time of metamorphosis and only acquire full development long after transformation when they come to resemble to a striking degree the oviducts of females (Fig. 2). In the larva of *R. pipiens* the so-called

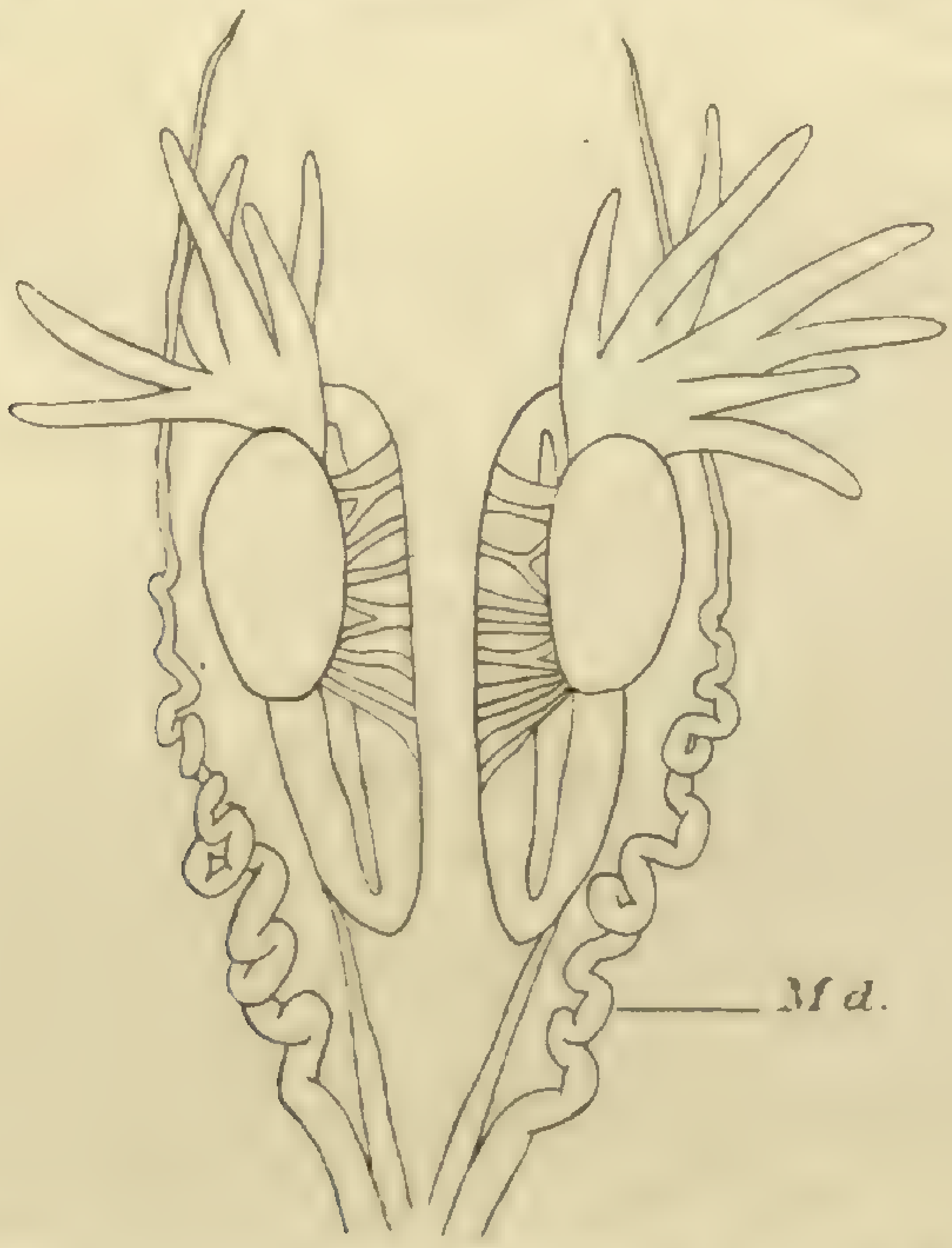


FIG. 2. Urogenital apparatus of adult *Rana pipiens* showing the normal condition of the Müllerian ducts (*md*) in males of this species.

transformation of females into males (degeneration of the pro-testis and formation of the definitive testis) occurs very early in larval life, before the Müllerian ducts appear, and in this species the ducts undergo practically their entire development after the definitive testis has formed. In other words, while subjected to the influence of the fully formed testis and its ripening sex products the ducts undergo the most marked development known in the males of any anuran species. Moreover, in *Rana catesbeiana,* where if we accept Witschi's interpretation of femaleness, the so-called transformation of female in-

dividuals into males is a prolonged process requiring two years, and where the future male larvæ are subjected to the so-called female influence during the entire period, the Müllerian duct does not appear. At metamorphosis when the definitive testes are fully formed and spermatozoa are beginning to appear the cellular cords representing the vestigial Müllerian ducts of the male form but do not develop. If Witschi's interpretation were correct, one would certainly expect to find marked development and hypertrophy of the Müllerian ducts in *R. catesbeiana* because of their being so long exposed to female influence. As a matter of fact, these structures in the male bullfrog are less developed than in other forms.

The same criticism applies to the so-called developmental correlation of the Müllerian duct with the gonad of the same side in cases of lateral hermaphroditism. What Witschi terms lateral hermaphrodites are nothing more than larvæ or young frogs which show the definitive testis developing out of the pro-testis (larval male Bidder's organ) faster on one side than on the other. (See Witschi, AM. NAT., page 533.) In the end such animals develop into definite males with testes symmetrically formed. True lateral hermaphroditism in adult frogs is an exceedingly rare phenomenon. In the writer's material it is rare to find both definitive testes developing out of the pro-testes at the same rate, one gland may be the finished gonad, the other the pro-testis undergoing degeneration. Such larvæ are in no sense to be regarded as lateral hermaphrodites. There is no developmental correlation of the Müllerian ducts with the gonad of the same side in *R. catesbeiana* and *R. pipiens*, because there are no ducts formed until after the definitive testes are formed. Regarding the other somatic sex characters such as seminal vesicles and thumb cushions, it should be pointed out that the thumb pad in *R. catesbeiana* is not formed until after metamorphosis when

PLATE I

FIG. 3. So-called oocytes occurring in the degenerating pro-testis of larval bullfrogs. These cells according to the writer's view are merely hypertrophied spermatocytes that have undergone oviform degeneration.

FIG. 4. Section of pro-testis male larva before onset of degeneration. At *X* is spermatocyte in prophase. The black bodies are ring tetrads.

FIG. 5. The giant spermatocytes of *Scolopendra Heros* (Chilopoda). These cells form functional spermatozoa and make up the greater part of the testes. Note the "germinal vesicle" condition of the nucleus.

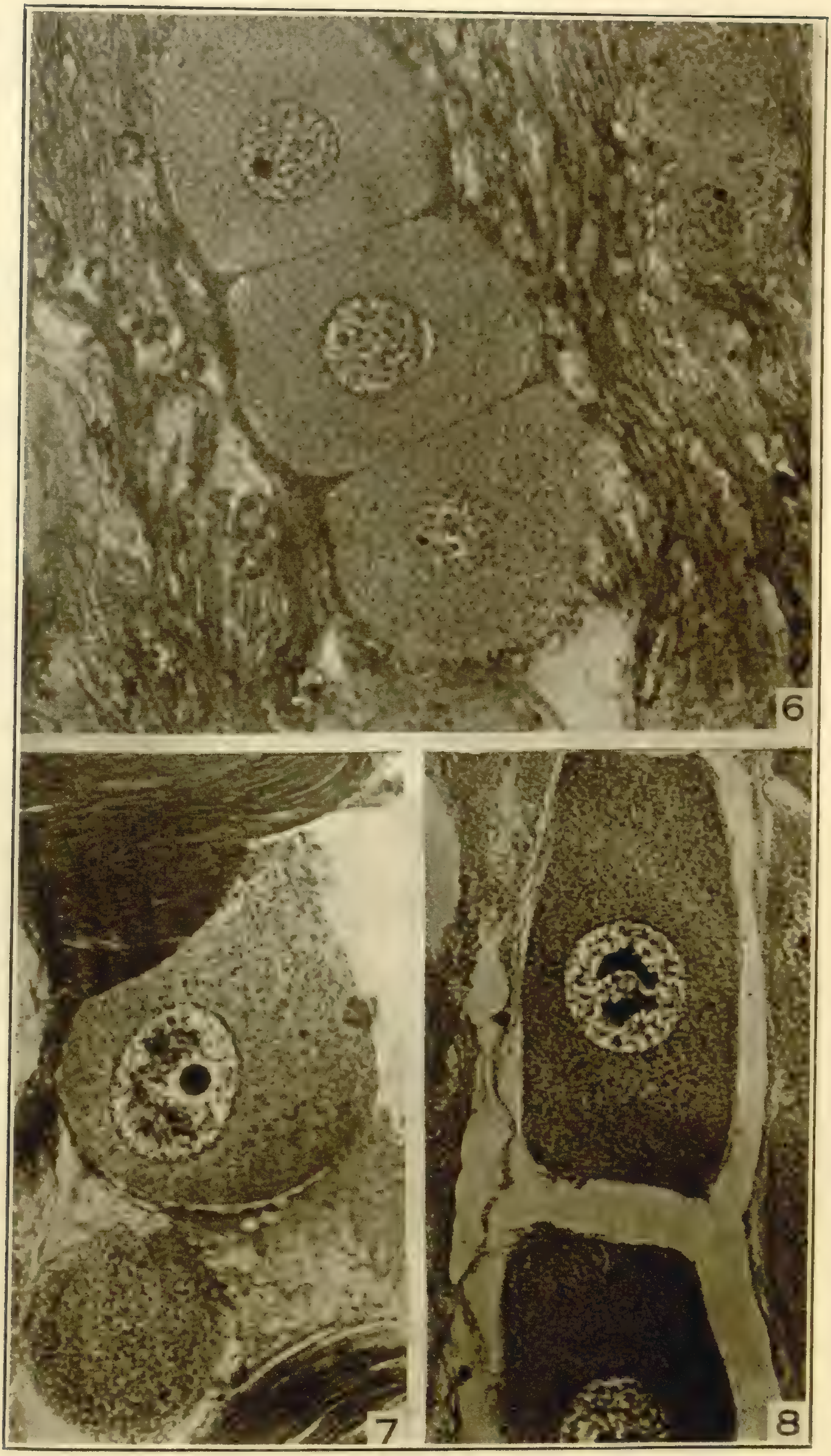


PLATE II

FIG. 6. Spermatocytes of *Scolopendra.*

FIGS. 7 and 8. Spermatocytes of *Lithobius* (Chilopoda). The resemblance to oocytes in the germinal vesicle stage is remarkable. Sections of the testes look like ovaries.

All photographs on Plates I and II made at a magnification of 500 diameters. No reduction. Figures 5–8 are from Professor Blackman's material.

the fully formed testes are present, and the seminal vesicles are absent or rudimentary in the males of many frog species, and exceedingly well developed in others.

In the few cases reported of true lateral hermaphroditism in adult frogs there is *not always* a developmental correlation of the Müllerian ducts with the gonad of the same side. Crew ('21), (*Journal of Genetics*, Vol. II, no. 2) has summarized the known cases of sexual abnormality in amphibians — see Figs. 7, 8, 9, 12, 14, and 16 of this paper, also the report of cases 21, 22, 23, 24, and 39. These are exceptions to any rule of developmental correlation. In several cases, Figs. 25 and 31, the ducts are quite as well developed in total absence of ovarian tissue as when such is present in large amounts, this, of course, being the normal condition in *Rana pipiens*. Crew also gives a list of frog cases reported where both gonads were entirely missing and yet the Müllerian ducts were well developed in such individuals.

Because of these facts it is fair to conclude that the appeal to the somatic sex characters completely fails as proof of the transformation of female frogs into males.

In closing, it should be pointed out that Witschi has made but one original investigation of sex in anurans (Witschi '14, no. 1). His later papers on the subject contain no new observations or experiments but are purely speculative endeavors to interpret his early work in accordance with Mendelism ('14, no. 2), later ('20, no. 3) in accordance with internal secretions.

THE SEX-LINKED GROUP OF MUTANT CHARACTERS IN DROSOPHILA WILLISTONI

REBECCA C. LANCEFIELD AND CHARLES W. METZ

Station for Experimental Evolution, Cold Spring Harbor, N. Y.

Introduction

The present work was undertaken for the purpose of comparing the genetical behavior of the fruit-fly *Drosophila willistoni* with that of *Drosophila melanogaster* and other members of the genus. It deals with the 28 sex-linked mutant characters thus far studied. The non-sex-linked characters will be considered in another paper.

Drosophila willistoni Sturtevant (*D. pallida* Williston)[1] is not unlike the well-known *D. melanogaster* in habits and superficial appearance, but a detailed examination reveals numerous features in which it differs from *melanogaster*. Among these are the following: (1) absence of sex combs in the male, (2) six instead of eight rows of acrostichal hairs on the thorax, (3) smaller size and more slender form, (4) vermilion instead of red eye color, and (5) narrow instead of broad bands on the abdomen.

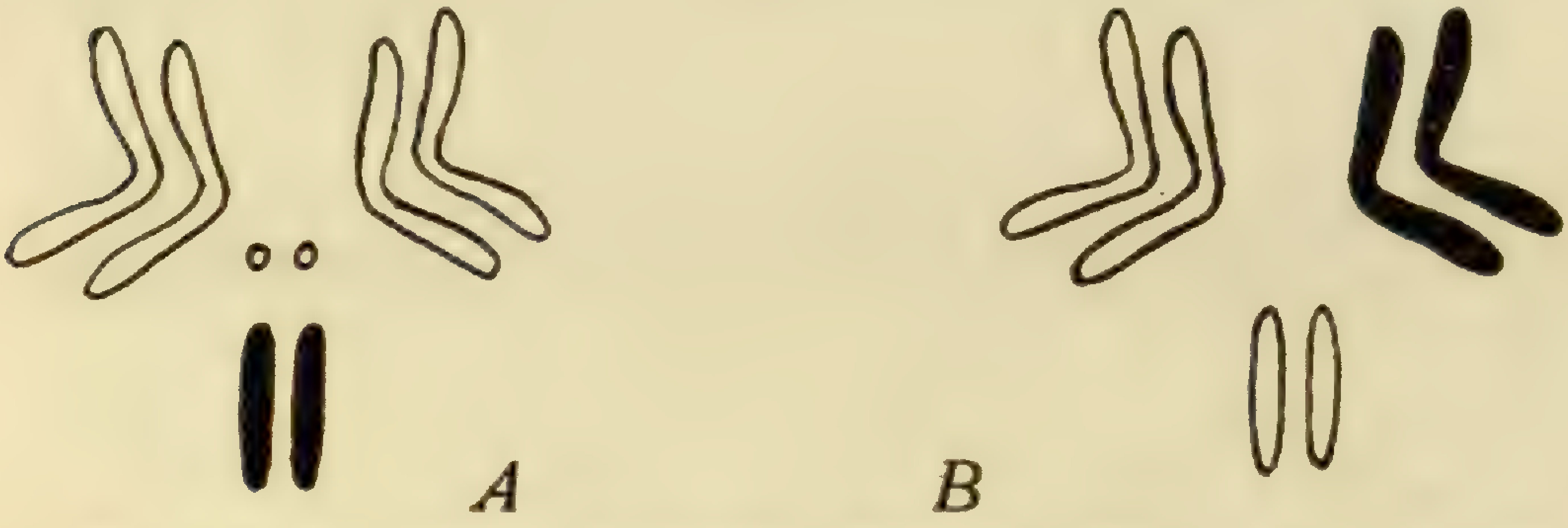
This species has been chosen for the present study because it is one of the species having the same general type of chromosome group as *D. melanogaster*. It will be recalled that within the genus Drosophila at least eleven different types of chromosome groups are represented (Metz, 1916). The most common type is that called type A (Fig. *A*, present paper), which is found in 13 of the 29 species studied. In these 13 species (which include *melanogaster* and *willistoni*), the chromosome groups are so much alike as to suggest that similar chromosomes are homologous and carry homologous groups of genes throughout. On the other hand, the species themselves do not form a restricted taxonomic group, but seem to be

[1] See Sturtevant, 1921*b*, for description, etc.

scattered more or less at random through the genus—which does not conform to such a view unless this type of chromosome group be considered primitive and the forerunner of several other types.

These considerations indicated the need of a comparative study of different species possessing this type of chromosome group, in addition to the studies already being made on species having different types of groups. Since the species can not be hybridized (or have thus far refused to hybridize—with one exception considered below), it is necessary to make cytological and genetical studies of them individually. This of course limits the comparison to a very few species.

The present paper supplements our previous one (Lancefield and Metz, 1921) on the sex chromosome relationships of *willistoni* and *melanogaster,* in which it was shown by means of non-disjunctional flies that the sex chromosomes are not strictly homologous in the two species. In *melanogaster* the short, rod-like pair is the sex chromosome pair (Bridges, 1916), whereas in *willistoni* we find that the rod-like pair is an autosome pair, and that one of the large V-shaped pairs is the sex chromosome pair. This relationship is shown in Figs. *A* and *B*.



FIGS. *A* and *B*. Diagrams of female chromosome groups of *Drosophila melanogaster* and *Drosophila willistoni* respectively. The X-chromosomes are represented in solid black, the autosomes in outline. In *D. willistoni* the small, dot-like pair may be absent.

The genetical study considered here is for the purpose of comparing the constitution of the sex chromosomes by

means of the sex-linked characters and their linkage relations.

The stock of *D. willistoni* which we have used was brought from Cuba in 1915, and was kept in the laboratory without being studied until 1919 when the present work began.

In making the tests for linkage and in calculating crossover values, the usual procedure has been followed.[2] We have been concerned particularly with determining the relative order of the genes and the approximate amount of crossing over between them, but not with obtaining exact crossover values. In consequence, the " chromosome map " given here is to be considered as indicating only the approximate location of the respective genes.

In presenting the data, the mutant types are described, not in chronological order, but in such a way as to follow the serial order of the genes on the chromosome map. All of the sex-linked characters are recessive. The data on the origin of mutants are necessarily imperfect, and in some cases are very meager, owing to the fact that many of the mutants appeared in stock cultures, mass cultures, etc., for which no complete records were taken. In such cases the available data are given briefly under the appropriate headings.

We are indebted to Mr. D. E. Lancefield for carrying out some of the early experiments, and for finding the mutants "rimmed" and "nicked." Similarly, we are indebted to Miss Ruth Ferry for the mutant " yellow " and for carrying out the experiments involving " yellow." To Dr. A. H. Sturtevant we owe many valuable suggestions regarding the comparison of mutant characters in *D. willistoni* with those in *D. melanogaster* and *D. simulans*. We are also indebted to the following persons for making the accompanying drawings: Miss Ruth Lincks — Figs. 1, 3, 7, 8; Mrs. D. B. Young — Figs. 2, 4, 5, 6, 17; Miss E. M. Wallace — Fig. 9, and Miss E. D. Mason — Figs. 10–16.

[2] This has been described in earlier papers by Morgan and others, and is to be found in current books on genetics.

DESCRIPTION AND ORIGIN[3] OF MUTANT CHARACTERS

Stubby (*sy*)

Description.—Stubby is a bristle character, manifested by all of the thoracic and head bristles (Fig. 2). These are usually shortened, thickened, and somewhat curled, and often are split or forked at the tip. The two posterior scutellar bristles are frequently tightly twisted together and point anteriorly. The short, thick appearance

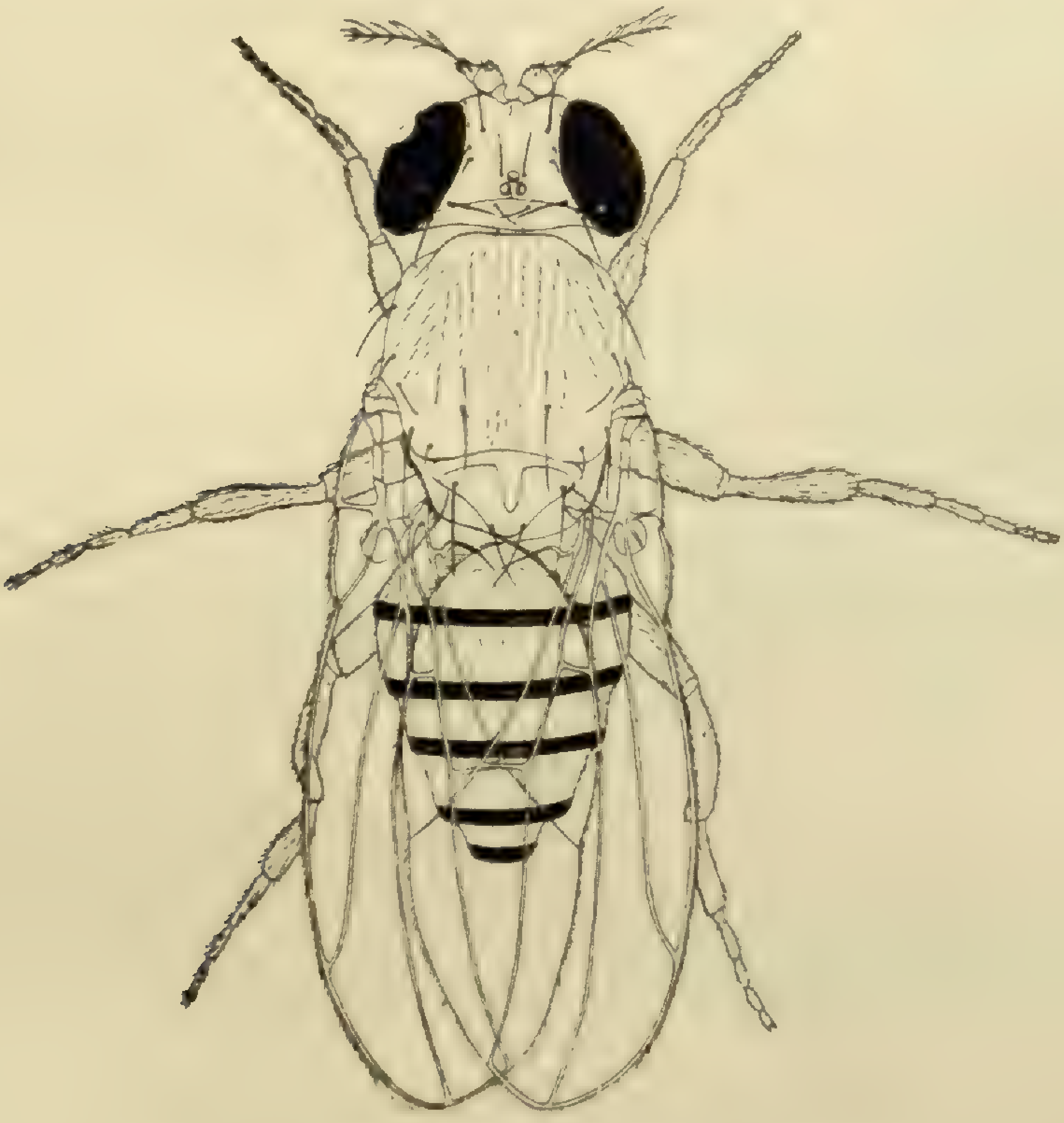


FIG. 1. *Drosophila willistoni,* "normal."

of the bristles is never apparent in combination with small-bristle, but the character can always be distinguished by the forking of the sternopleural bristles. Both sexes are fertile. Stubby looks very much like forked in *melanogaster.*

Origin.—One stubby male was obtained from a pair mating. No complete record of this culture was kept, however, and it is not known whether others appeared previously or not.

[3] See Table I.

Orange (*o*)

Description.—The eyes are orange colored. In newly hatched flies, the color is a pale lemon, which deepens into orange as the fly matures and may become very dark in old age. The color resembles garnet or coral of *D. melanogaster.*

FIG. 2. Stubby, bristles.

Origin.—One male appeared among the offspring of a mating of three normal females by an unrelated male. The other offspring were all normal, but their number was not recorded. Presumably, the mutation occurred in one of the P_1 females and affected only one or a few germ cells, although it is possible that this female was heterozygous and produced a very small number of the flies in the culture.

Small-bristle (*sb*)

Description.—All the bristles are more slender and somewhat shorter than in normal flies. The character is extreme when orange eye color is also present.

Origin.—One small-bristle, forked male was obtained from a mass culture carrying rough and forked.

More than a year later, a second mutation to small-bristle occurred in an entirely unrelated stock. In this case the single small-bristle male found among the offspring of one pair was crossed to a female from a homozygous orange small-bristle stock and produced only small-bristle flies.

Bent (*bn*)

Description.—The wings of bent flies are slightly spread out, and are bent at the base so that they slope down toward the body (Fig. 3). They are often slightly crumpled. The flies hatch as well as their normal sibs but do not breed as readily.

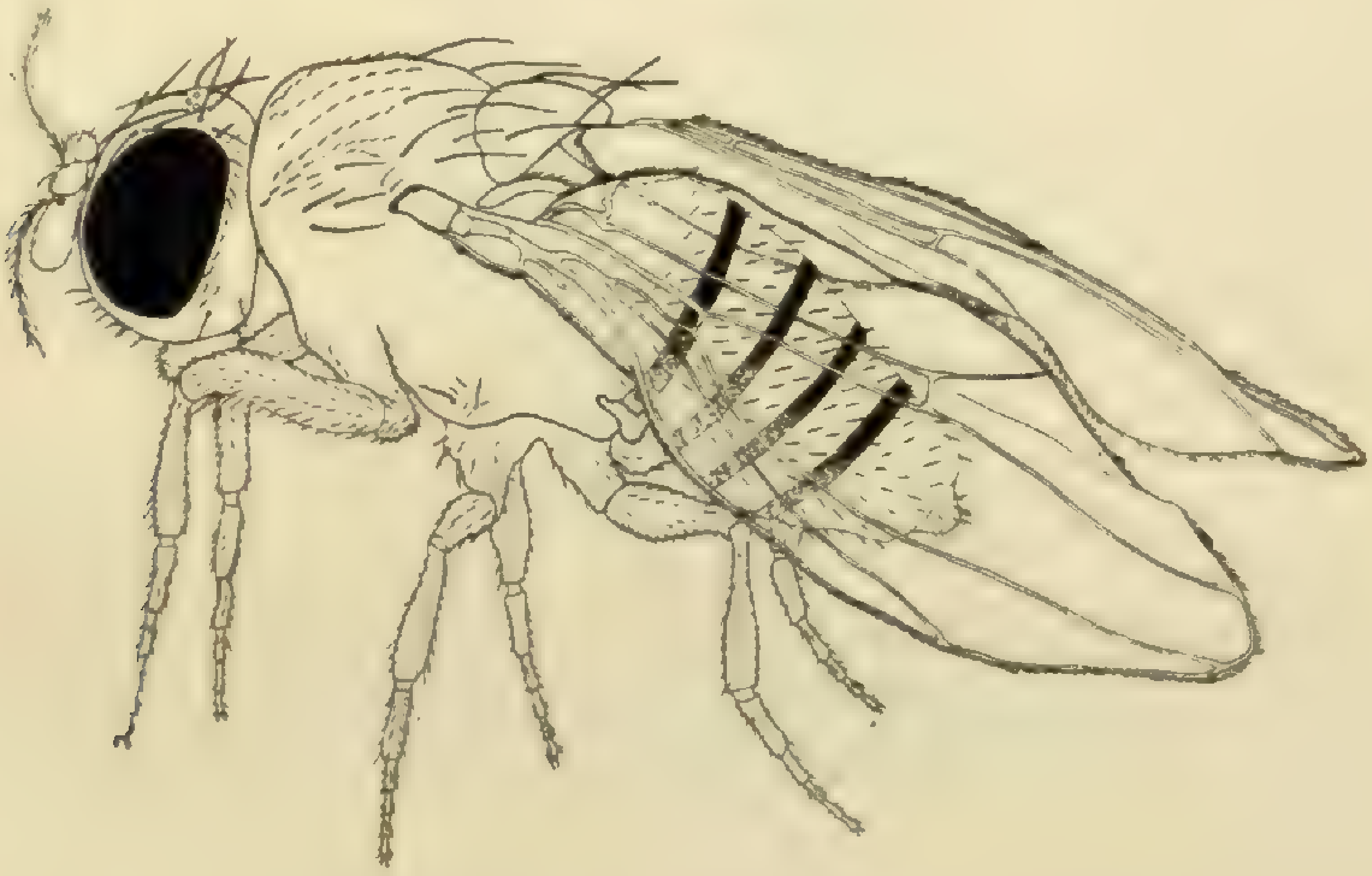


FIG. 3. Bent, wings.

Origin.—Many bent males were found in a culture of five orange females from stock mated to a single male of different parentage. At least one of the females carried bent, but the exact origin is uncertain. No bent flies were ever observed in orange stock.

Forked (*f*)

Description.—In forked flies, all the bristles are wavy with the ends sometimes forked. The females are sterile. This character is similar to, but less extreme than, stub-

by. It recalls singed$_2$ of *melanogaster* although singed$_2$ is slightly more extreme.

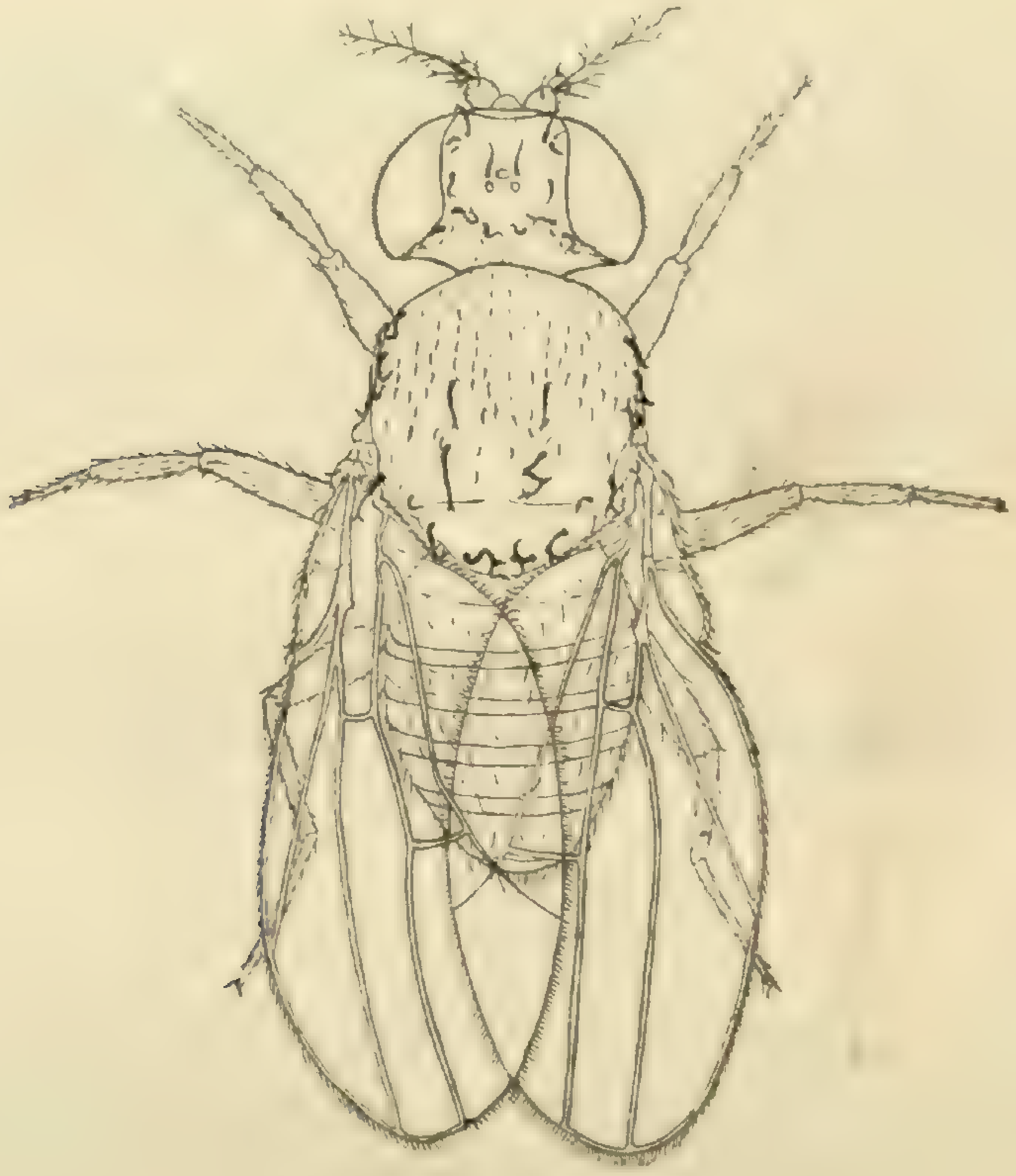


FIG. 4. Forked-2, bristles.

Origin.—Seven males were found in a stock bottle. Since no forked females were obtained, it is possible that this was the original appearance of the mutant and that all of the forked flies were from one mother, heterozygous for forked.

Forked-2 (f_2)

Description.—This character is much more extreme than its allelomorph, forked, or the similar mutant, stubby. The bristles are twisted and thickened with their ends often split (Fig. 4). The twisting also affects practically all the hairs on the fly, including those on the inner margin of the wing. The hairs on the antennæ are forked. Forked-2 resembles the *melanogaster* singed. The females are sterile.

Origin.—Several forked-2 males and one female appeared in a stock bottle of small-bristle flies.

Tiny (*t*)

Description.—Tiny-bristle flies usually have small anterior dorso-central bristles. Sometimes the two anterior scutellar bristles are also small. Occasionally all the bristles may be small, so that the individual may be indistinguishable from a true "small-bristle" fly. The character is rather variable and, in many cases, is very hard to separate from normal. This difficulty was so great that the stock was finally discarded.

Origin.—A single male was found on the last count of the offspring from a pair mating. It is possible that other tiny males were present among the previous offspring and escaped observation since the character is very inconspicuous.

Square (*sq*)

Description.—The wings are about two thirds the normal length with the ends almost square instead of pointed (Fig. 11). A characteristic slight wave extends throughout the length of the wing. The females are sterile and the viability of the males is rather poor.

The description of square suggests that of rudimentary *melanogaster* but square is much less extreme than rudimentary; the wing is not shortened so much and is not cut off so squarely.

Origin.—Among the offspring from a pair mating (rough female by orange rough stump male) several square males were found, indicating that the mother was probably heterozygous for square.

Reduced (*re*)

Description.—Reduced flies regularly lack the two anterior dorso-central bristles; occasionally, they also lack one of the posterior dorso-centrals; and less frequently, all four are absent. In combination with scute-2, however, the more extreme condition of reduced is frequently found (Fig. 7). The reduced gene also affects the shape

of the abdomen, which is blunted, or apparently compressed along the anterior-posterior axis. The abdominal bands are slightly irregular.

Reduced flies, especially females, are hard to breed in pairs, although those which do produce offspring seem normally fertile.

Origin.—Many males were found among the offspring from a pair mating from orange stock. The mother of the culture was apparently heterozygous for the gene.

The Scute Allelomorphic Series

1. *Scute* (*sc*)

Description.—The two anterior scutellar bristles are usually lacking, although occasionally only one may be gone. Rarely the combination of one anterior scutellar bristle and one posterior one may be found. The remaining bristles are normal in size. The character almost always manifests itself in homozygous flies. Only one exception to this has been detected up to the present time.

Origin.—Fifteen scute males and eleven normal males were obtained from a normal pair. The female offspring were all normal (number not recorded). It is almost certain that the mother was heterozygous in this case, and that the mutation occurred in a previous generation or else very early in her own ontogeny.

2. *Scute-2* (sc_2)

Description.—Scute-2, an allelomorph of scute and scute-3, involves the same scutellar bristles as scute, but varies toward a more extreme condition than this allelomorph. The two anterior scutellar bristles are always missing, frequently one of the posterior scutellars is gone, and occasionally all four are lacking. The bristles on the scutellum are fine and small. In a stock homozygous for reduced and scute-2, both characters are more extreme than either is alone (Fig. 7). Such flies often entirely lack dorso-central and scutellar bristles, and lack one or more orbital bristles.

The compound scute scute-2 females are either somewhat intermediate between the two, or they may look entirely like one or the other component. On the whole, they are more apt to resemble scute-2 than scute.

Origin.—From a normal female mated to a scute male, the following types of offspring were obtained: males — one half normal, one half scute-2; females — one half normal, one half somewhat intermediate between scute and scute-2. From this it was concluded that the parent female was heterozygous for the new character and that this character was allelomorphic to scute, a conclusion subsequently verified by direct tests. Six sisters of this female were also tested and none gave scute-2.

3. *Scute-3* (sc_3)

Description.—Scute-3 is an allelomorph of scute and scute-2. All four scutellar bristles, the two sterno-pleural bristles, and a varying number of head bristles are absent. On the head, all three pairs of orbitals are usually missing, and occasionally some of the others are gone.

The compound females involving scute-3 and scute-2 are more apt to be like scute-3 than like scute-2, although in general they are intermediate. Such females could be distinguished from the homozygous scute-2 females in all the cases observed by the absence of at least one sterno-pleural bristle and generally by the absence of all scutellar bristles. Scute-3 males are sterile.

Scute-3 strongly resembles the scute of *melanogaster*. In both cases scutellar and head bristles are affected. Two stocks of *melanogaster* scute kindly examined by Dr. Sturtevant agree with scute-3 in lacking scutellar bristles and orbitals, and in having small ocellar bristles. They both possess post-orbitals, however, and one stock occasionally shows the middle orbital present. The other usually lacks the postverticals.

Origin.—Scute-3 was first observed in the offspring of an F_2 female from a cross of a scute female from stock by two rough rimmed stump males. This female seemed to

be heterozygous for the new factor, and it was found that the character was also present in males in sister cultures which had been used for stocks.

Yellow (*y*)

Description.—In " yellow " flies the body, wings and legs are deep yellow. The bristles and hairs are all yellowish or bronze instead of black. In the latter respect yellow differs from the yellow in *Drosophila virilis* which has black or dark brown bristles and hairs.

Origin.—A single yellow male appeared in a bottle of scute rough stump stock.

Yellow was found after the main part of this paper was prepared for publication, and since the experiments involving it have not added materially to the data given in the tables they are omitted from the latter and are given briefly here.

The original yellow scute rough stump male was mated to normal females giving a normal F_1. The latter, inbred in pairs, gave 1354 normal daughters and the following classes of sons: normal 488; yellow scute rough stump 466 (non-crossovers 954); yellow scute 25, rough stump 23 (single crossovers in region two 48); yellow scute rough 49, stump 46 (single crossovers in region three 95); yellow scute stump 3, rough 2 (double crossovers involving regions two and three, 5). In addition, two yellow rough stump males and one yellow stump male were obtained. Of the former, one proved to be genetically scute when tested and hence should be in the non-crossover class. The other gave no progeny, but presumably was also a non-crossover. The third fly likewise failed to breed, but since it lacked rough as well as scute it presumably represents a double crossover in regions one and three. It is this fact which leads to the tentative location of yellow above rather than below scute on the map.[4]

[4] This is supported by subsequent data.

Peach (*p*)

Description.—Peach eye color is practically indistinguishable from orange eye color, although, as a rule, it is a trifle darker than orange and does not have the range of shades due to age which are observed in orange. In the same culture, it is impossible to distinguish the two with certainty. The double recessive of peach and orange is probably indistinguishable from either eye color alone. Homozygous peach rough flies have darker eye color than orange rough flies, and are hard to separate from rough alone. Peach eye color is similar to ruby and garnet of *melanogaster* and to rubyoid and carmine of *simulans*.

Origin.—A single male with peach eye color was found in a double recessive forked stump stock.

Beaded (*be*)

Description.—Beaded refers to the condition of the wings, which have the marginal hairs clumped in irregular patches, especially on the posterior half of the outer margin. The wings are pointed at the ends due to a long notch, extending from the tip of the third vein to about

5

6

FIG. 5. Normal, wing.
FIG. 6. Beaded, wing.

the region of the posterior cross-vein, and to the loss of a section from the outside of the wing between the distal ends of the second and third veins (compare Figs. 5 and 6). Beaded flies have poor viability, and the females are sterile. Beaded is similar in appearance to the *melano-*

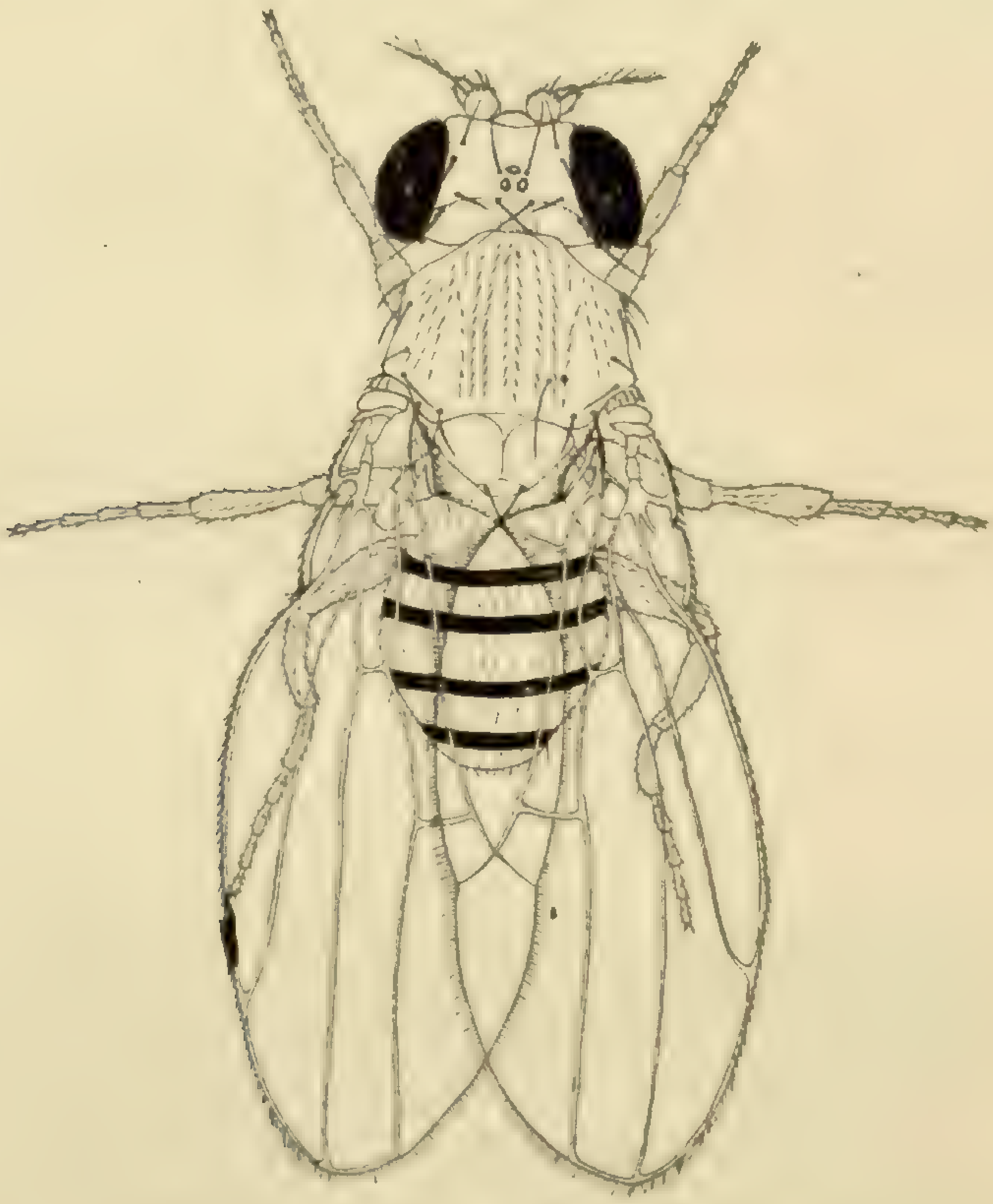


FIG. 7. Reduced scute-2 compound.

gaster cut_6 although the latter is slightly more extreme than beaded. While cut_6 flies are vigorous and fertile, some of the cut allelomorphs are not completely fertile and have poor viability.[5]

Origin.—An out-crossed female, known to carry small-bristle rough on one X-chromosome, and small-bristle orange short-3 on the other, produced offspring in which the small-bristle rough males were also beaded. This female was almost certainly heterozygous for the new gene. Nine sisters were bred separately but no beaded flies appeared in their offspring.

[5] Unpublished data for which we wish to thank Drs. Mohr and Bridges.

Rough (*r*)

Description.—Rough eye affects mainly the surface of the eye (Fig. 8). When the outer portion of the eye is mounted and examined under the high-power microscope it is seen that the ommatidia are irregular in shape and size with uneven surfaces which are more convex than the normal. The normal eye has regular hexagonal facets with a bristle at every alternate intersection of the sides (See Carnegie Publ. 278, Plate 10, Fig. 3*c* for the normal eye of *D. melanogaster* which has the same arrangement). The bristles of rough eye are irregularly distributed with groups collected in one place and no bristles at all in another. These bristles are about one and a half times the length of the normal ones.

The roughened condition is similar to that found in star eye of *melanogaster* (Carnegie Publ. 278, Text-figure 83). The eyes of *willistoni* rough are also somewhat glossy in texture, and the wing veins are slightly heavier than in the normal flies.

Origin.—Several rough males and females were found in one of the bottles of a stock that had been kept in the laboratory for approximately four years. It is probable that the mutant gene had been present in the stock for some time.

Triple (*tr*)

Description.—Triple causes four variable wing changes, one or all of which may be present in either or both wings (Fig. 10). (1) The second and third veins may be fused for a short distance at their origin. (2) The wings, slightly tilted up at the ends, are held away from the body at an angle which varies up to about 90°. (3) The third veins fail to reach the distal margin of the wings by amounts which vary from almost nothing to one third the length of the vein. This is particularly evident in the females, where a large section may be missing from the central part of the third vein. (4) An extra cross-vein is present between the second and third

veins at a level about half way between the anterior and posterior cross-veins. This vein, when not wholly formed as a cross-vein, is often indicated by short pieces of disconnected vein.

The fusion of the second and third veins and the extension of at least one wing are constant characters as far as observed. The latter forms the easiest basis of distinguishing this mutant. The females are more extreme than the males in all four of the changes involved. Triple suggests the *melanogaster* mutant, bifid, by its extended wings, fusion of the veins at the base of the wing, and the shortening of one of the veins, although the short vein is the fourth in bifid and the third in triple and the third vein of bifid is thickened at the distal extremity.

Origin.—Triple was first noticed in the offspring of a female out-crossed from wild stock. Half the males were triple. On investigation it was found that several bottles of wild stock contained similar males.

Triple males were found six months later in stump stock. Crossed to the original triple stock, these males produced triple female offspring in the F_1 generation. The possibility of contamination of the stump stock can be eliminated since the triple males found in the stock were also stump, and there were no cultures containing triple stump flies in the laboratory.

The Deformed Allelomorphic Series

1. *Deformed* (*d*)

Description.—Deformed, which involves many parts of the body (Fig. 9), shows sexual dimorphism. In the male the eye is about two thirds the normal size and very rough; in the female the eye is normal in size and only slightly roughened. In both sexes the bristles are abnormally long and irregularly bent. The thoracic hairs are badly disarranged in both sexes, but the effect is very much more exaggerated in the female than in the

male. In the former, the hairs are often clumped in a compact mass on the anterior half of the thorax; while in the male, the hairs are irregularly scattered over the

FIG. 8. Rough (eye), rimmed (wing margin), stump (second wing vein) compound.

whole area. The scutellum in the male (sometimes in the female also) is blunt instead of pointed, posteriorly, and the under portions of the thorax are consequently visible. The wings are extended at an angle of about 45° to 90° in both sexes, and the veins are often faint, short, and irregular, especially in the male.

These flies are rather feeble and breed poorly except in mass culture, probably on account of the many physical defects present.

Origin.—Many males and females were found in a stock culture of orange forked rough. Sister bottles made up at the same time did not produce any deformed flies.

2. *Serrate* (*st*)

Description.—Serrate is an allelomorph of deformed, but involves only a part of the characters modified by deformed. The changes in the eyes of the two sexes are exactly the same as those caused by deformed. On the other hand, the bristles and the shape of the scutellum are almost normal and the thoracic hairs are only slightly disarranged. The wings may occasionally be held at an angle with the body, but the venation is practically normal. The only strikingly noticeable effect of serrate is the change in the eyes.

Fig. 9. Deformed ♂.

The compound deformed-serrate females are intermediate between the two allelomorphs but tend to resemble serrate more closely than deformed. Serrate flies are more viable than deformed and breed more readily.

Origin.—A single male was found in an F_2 mass culture from a mating of two females by one male from scute stock. No other serrate flies appeared in this culture or in a sister culture.

Rimmed (*ri*)

Description.—In rimmed flies, a heavy rim of marginal hairs surrounds each wing and the wings curve down

over the abdomen as if the margins were constricted (Fig. 8). The depression between the scutellum and thorax of normal flies is eradicated, leaving a smooth surface at the junction. The thick marginal rim of hairs is the most constant of the effects of rimmed, but the other changes are usually apparent.

Origin.—Several males were found in wild type stock.

Pale (*pa*)

Description.—The post-vertical and all the thoracic bristles are pale yellow. Occasionally, a few other head bristles are also yellow. The bristles are thin, and the entire fly is weak and small with the wings often not unfolded. None of the original pale flies could be induced to breed. The heterozygous females produced a few pale offspring, but the stock was soon lost.

Origin.—The mother of the culture in which pale appeared was heterozygous for scute rimmed on one X-chromosome and for pale morula on the other. Five sisters of this female were bred, but no pale offspring were obtained from any of them. It is impossible to tell whether the mutation to pale occurred in the mother of the culture in which pale was found or whether it took place in her mother.

Stump (*s*)

Description.—The distal portion of the second vein is lacking, leaving only a stump at the base of the wing (Fig. 12). This stump varies in length from one quarter to two thirds that of the normal vein.

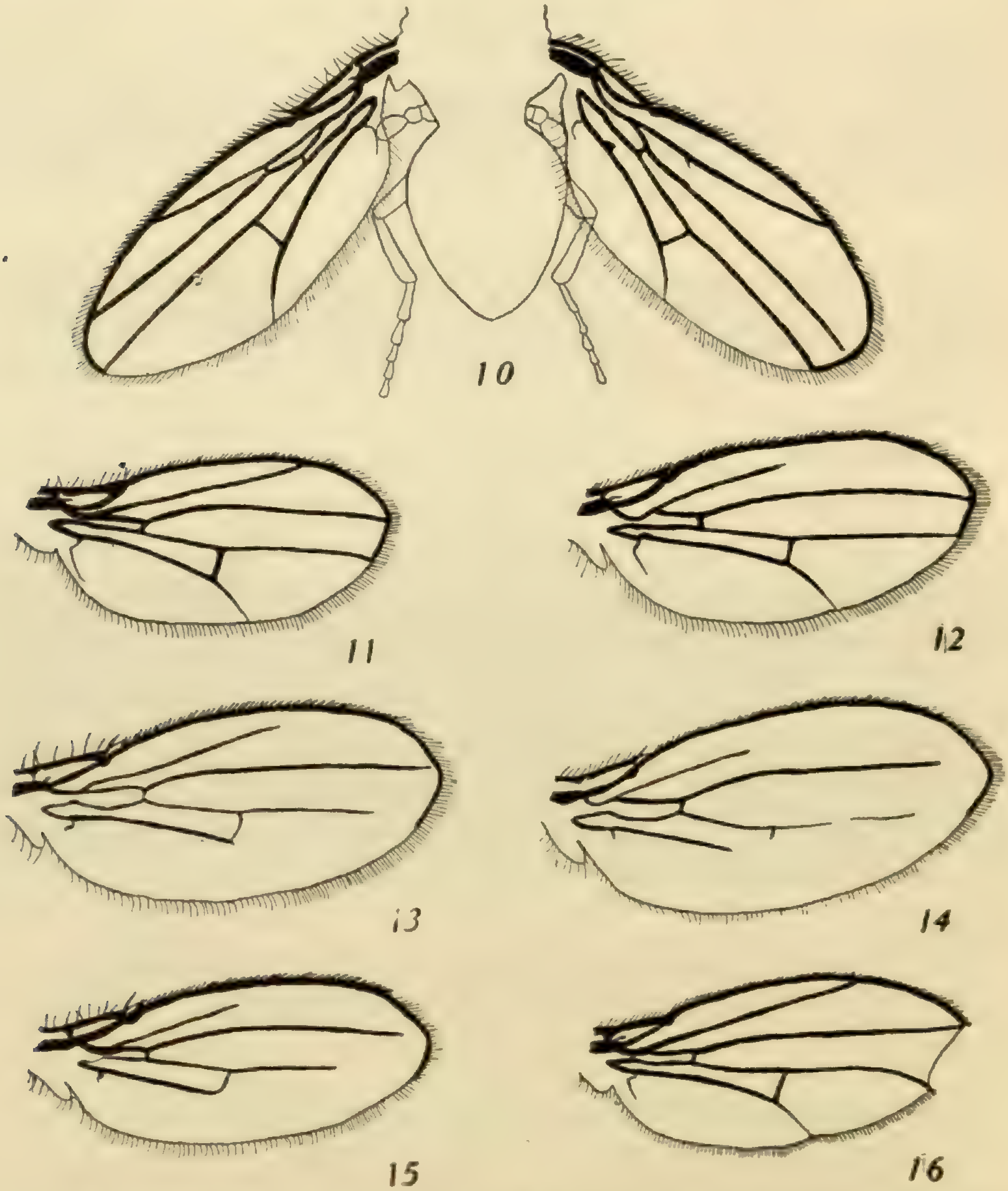
Origin.—Six stump males were obtained from a mass culture in which the mothers were heterozygous for forked and the fathers were normal. Four of the stump males were also forked, the others were not.

THE SHORT ALLELOMORPHIC SERIES

1. *Short* (*sh*)

Description.—Typically, all the veins of the wing fail to reach the margin in short flies, although

sometimes the third vein is entire (Fig. 13). The second vein is shorter than any of the others. Ordinarily, the distance between the ends of the veins and the margin is not great. The posterior cross-vein is broken occasionally.



FIGS. 10–16: Fig. 10, triple. Fig. 11, square. Fig. 12, stump. Fig. 13, short ♂. Fig. 14, short-2 ♂. Fig. 15, short-3 ♂. Fig. 16, nicked.

Origin.—Short was first observed in half the sons of a single female indicating that the mother was heterozygous for the short gene. This female carried orange

rough on one X-chromosome and stump on the other. The mutation to short evidently affected a locus of the orange rough chromosome not far from the stump locus. Several sister cultures were examined, but no short flies were found.

2. *Short-2* (sh_2)

Description.—Short-2 is the most extreme of the series. In the females the second, fourth, and fifth veins are very short, the fourth and fifth often not reaching as far as the posterior cross-vein. In cases in which they extend beyond the cross-vein, this vein is broken. In the males the fourth and fifth veins are about three quarters the normal length, and the posterior cross-vein is broken (Fig. 14). The males are indistinguishable from those of short-3.

Origin.—A single male was found in small-bristle rough stock.

3. *Short-3* (sh_3)

Description.—Short-3 is about the same in both sexes (Fig. 15). The second vein is very short, and all the others are about three quarters the normal length. The posterior cross-vein is usually broken.

Females containing any two of these three allelomorphs are intermediate between the two used, with perhaps a closer resemblance to the more extreme member of the pair.

Origin.—Several males were found in scute stock.

Morula (*m*)

Description.—Morula involves a partial roughening of the eyes which is due to a consolidation of a group of facets, especially in the central area of the eye, suggesting the lozenge of *melanogaster*. The viability of this stock is very poor, and the double recessives of morula and any other mutant character rarely survive.

Origin.—At least five males were obtained from a mass culture of three pairs which carried rough. The classi-

fication of rough and morula was not accurate at its first appearance.

Nicked (*nk*)

Description.—Nicked is characterized by irregular notches in one or both wings (Fig. 16). The indentations vary in size and location, but tend to show on the posterior and inner portions of the wing. In certain lines, the character shows regularly, while in certain other lines it overlaps normal a great deal. Flies in which nicked is combined with other mutant characters have rather poor viability.

Origin.—Several individuals of both sexes were found in a mass culture.

Rosette (*ro*)

Description.—In this mutant race, a large number of characters are affected (Fig. 17). The eyes are slightly roughened due to disarrangement of the facets; the thoracic hairs are disarranged, looking as if they had been brushed in the wrong direction; the bristles may be bent; the distal tarsi of the legs may be twisted; and the wings are generally held at an angle with the body, and one or both may be small and circular. The rough eyes and disarranged hairs are constant characters. Rosette flies have very low viability and are hard to breed, especially when other mutant characters are present also.

Origin.—Four rosette rough males were obtained among a large number of offspring from one morula male by three rough females.

Construction of the X-Chromosome "Map"

With one or two exceptions the usual procedure[2] has been followed in constructing the chromosome "map." The order of the genes was determined by means of crosses involving three or more loci (Tables III–VI), and that order adopted which, in the consideration of any three points, made the double crossover class the small-

[2] See footnote, p. 213.

est. In most cases the decision was confirmed by several subsequent experiments made for other purposes. Tiny and square have not been definitely located with reference to forked since they proved unsuitable characters for use in linkage experiments. Similarly, the loci of triple and deformed are known to be between rough and rimmed, but the relative positions of the two could not be determined on account of the impossibility of using the two characters together. Pale, morula, and rosette are also only tentatively placed.

FIG. 17. Rosette.

With the order of the genes established, the "distances," or crossover values, between them were obtained by combining the data from all the experiments given in Tables II–VI. Table VII gives the summary of all data between any two loci in the "map" from those experiments in which no intermediate genes were concerned. This differs from the usual method of summarizing the data in that it includes only experiments in which no intermediate point was used.

As far as possible the positions of the genes on the "map" have been determined by summation of the "distances" between neighboring loci taken in pairs, using stubby arbitrarily as the zero point. In several cases, however, the locus of a gene has been assigned by reference to some main well-established point; notably, orange, forked, scute, rough, or stump. In Table VII, the starred data are those on which the "map" is based. No correction for data involving non-adjacent loci has been made, since the degree of numerical accuracy does not warrant such a computation in this case. No correction has been made for double crossing over in long regions in which no mutant loci are known.

Owing to the possible parallelism between the yellow and scute in *willistoni* and the yellow and scute in *melanogaster* a second set of readings has been given on the "map" using the position of yellow as the zero point and plotting the others in opposite (+ and —) directions from this. Comparison with the X-chromosome map of *melanogaster* is thus facilitated.

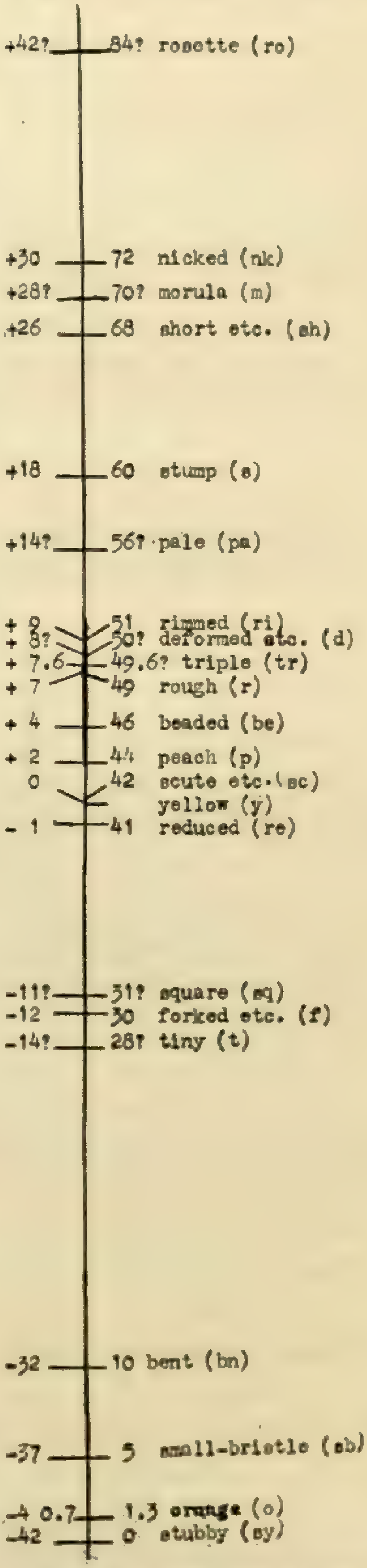


FIG. 18. Map showing linkage relations of sex-linked genes in *Drosophila willistoni*.

Discussion

The previous work on the comparative genetical study of different species of Drosophila has been concerned largely with species having different types of chromosome groups. It has involved mainly the species *melanogaster, virilis, funebris, simulans* and *obscura*. Of these, only *melanogaster* and *simulans* have the type of chromosome group with which we are concerned here. The published data on the first four of these species have recently been summarized by Sturtevant (1920) and may be passed over briefly. The data on *obscura* are in press and our references to them are made with the kind permission of Mr. D. E. Lancefield.

In *melanogaster, virilis, funebris* and *obscura*, the evidence suggests a tendency on the part of each species to give mutants paralleling those in the others, although the extent of this tendency can not be ascertained accurately because of the impossibility of proving the homology of similar characters. In the case of *melanogaster* and *simulans* the parallelism extends to nearly all of the known *simulans* characters and certain homologies are established by means of hybridization (Sturtevant, '20, '21*a*, 21*b*). To be sure, the two latter species are almost identical and would be expected to give similar genetical results; but it is of interest to note that there is a close resemblance between the proven cases of parallel characters in these, and the apparent cases of parallel characters in the other species. This tends to increase the probability of actual parallelism in the latter where a series of linked characters is involved.

Upon comparing the mutant characters of *willistoni* with those of the others it is evident that only a few striking cases of resemblance are found. Of these the most significant involve the characters yellow and scute. Their morphological resemblances to the yellow and scute in *melanogaster* have already been noted in the

descriptive section. But the evidence for their being parallels is made particularly strong by the fact that their genes are completely or almost completely linked in both species. In *melanogaster* yellow and scute are located at the extreme end (zero point) of the chromosome map, while in *willistoni* they are approximately in the middle.

A situation similar to this has already been found in *D. obscura* (according to unpublished data of D. E. Lancefield). Here the characters yellow and scute also bear a close resemblance to those in *melanogaster* and are very closely linked. As in *willistoni,* the factors for yellow and scute are near the middle of the chromosome map. It will be recalled that *obscura,* like *willistoni,* has a large V-shaped X-chromosome—although the other chromosomes are different (Metz, 1916). In the two species having V-shaped X-chromosomes, then, yellow and scute are "located" near the middle of the chromosome map, while in *melanogaster* with its short, rod-like X-chromosome, yellow and scute are at one end. As Lancefield has pointed out in his discussion of *obscura,* this suggests that one end of the rod-like X in the one case corresponds to the middle of the V-shaped X in the other. And this suggests that the rod-like chromosome itself may correspond to one arm of the V.

The only evidence in *willistoni* on the latter hypothesis is that furnished by the characters forked and stubby. These are possible parallels of the singed and forked in *melanogaster.* They are similar in a general way in the two species (see description above), and the serial order of the genes is the same (Fig. 18), although the linkage relations do not agree exactly.

In this connection it may be recalled that "yellow," "singed" and "forked" have also been found in *Drosophila virilis* (Metz, 1918, and unpublished data), and may, likewise, be considered as possible parallels to those in *melanogaster. Virilis* has a rod-like X-chromosome resembling that of *melanogaster,* and the relative posi-

tions of the three genes on the chromosome map resemble those in *melanogaster.* Yellow is about three units from the end instead of at the end; singed is at about 35 instead of 21 and forked is at about 58 instead of 56.5.

The evidence is not sufficient to warrant the conclusion that these are actually homologous series, but the fact that such series exist suggests that by the present means it may eventually be possible to obtain reliable data for a comparison of the chromosomal make-up of the different species.

Among the other characters in *willistoni* which show some resemblance to characters in *melanogaster* or *simulans* the following may be noted as a matter of record, although there is little indication of their being actual parallels: orange and peach (which look alike) resemble coral or ruby; beaded is similar to the cut allelomorphs both morphologically and in respect to its sterile females and poorly viable males; triple suggests bifid, and morula looks like lozenge. The small bristle of *willistoni* may be comparable to the tiny bristle-2 of *simulans.*

The fact that the X-chromosomes in *willistoni* are morphologically like the large autosomes and not like the X-chromosomes of *melanogaster* suggests that we ought to compare, not only the sex-linked groups of the two species, but also the sex-linked group of *willistoni* with the non-sex-linked groups of *melanogaster.* This has been done, but without revealing any significant indication of parallelism.

In conclusion it may be noted that although the evidence is not yet clear on the genetic relationship of the sex chromosomes in *melanogaster* and *willistoni,* yet if the above suggestion is correct, that the X-chromosome of *melanogaster* corresponds to part of the X-chromosome in *willistoni,* then the resemblance between the chromosome groups of the two species is only superficial. It may also be noted that the genetic "map" of

the X-chromosome of *willistoni* at present is only slightly longer than the map of the *melanogaster* X-chromosome (84 as contrasted with 70 units), whereas the *willistoni* X-chromosome itself appears to be about twice the length of that of *melanogaster.* This suggests that crossing over is less frequent in *willistoni* than in *melanogaster.*

Summary

1. Twenty-eight recessive sex-linked mutant characters in *Drosophila willistoni* are described and their linkage relations considered.

2. In general, the genetic behavior of *willistoni* (as regards crossing over, etc.) is similar to that of *D. melanogaster* and the other species of Drosophila whose genetic behavior is known.

3. There is a strong indication of parallelism between the mutants yellow and scute in *willistoni* and yellow and scute in *melanogaster.*

4. In both species these characters are completely or very closely linked.

5. There is some indication of parallelism between the characters forked and stubby in *willistoni* and singed and forked in *melanogaster.*

6. In *melanogaster* the genes for yellow and scute are "located" at one end of the chromosome map, and singed and forked are 21 units and 56.5 units respectively from this end. In *willistoni* yellow and scute are near the middle of the map, and forked and stubby are on one side at 12 units and 42 units respectively.

7. Since the X-chromosome of *melanogaster* is short and rod-like, while that of *willistoni* is approximately twice as long and is V-shaped, this relation of the chromosome maps suggests that the *melanogaster* X-chromosome corresponds to one arm of the V-shaped X-chromosome of *willistoni,* with the locus of yellow corresponding in the two cases. This agrees with the suggestion made by Lancefield in the case of *D. obscura* in which the X-chromosomes resemble those of *willistoni.*

8. The comparative lengths of the X-chromosome maps in *melanogaster* and *willistoni* suggests that there is less crossing over in the latter than in the former.

TABLE I

ORIGIN OF MUTANTS

Explanation of "records": W indicates R. C. Lancefield records except numbers 1–100 which indicate D. E. Lancefield; L indicates C. W. Metz; R indicates Ruth Ferry.

Mutant	Symbol	First Found	Record	Parts Affected
1. Stubby	sy	March, 1920	W 1128	Bristles.
2. Orange	o	April, 1919	L 37	Eye color.
3. Small-bristle	sb	{ July, 1919 July, 1920	L 336 W 1745	Bristles.
4. Bent	bn	Nov., 1920	W 1687	Wings.
5. Forked	f	March, 1919	L 16	Bristles; fertility of females.
6. Forked—2	f_2	March, 1920	W 1177	Bristles, hairs; fertility of ♀♀.
7. Tiny	t	Jan., 1920	W 856	Bristles.
8. Square	sq	Feb., 1920	W 965	Wings; fertility of ♀♀.
9. Reduced	re	Oct., 1919	W 288	Bristles; abdomen.
10. Scute	sc	May, 1919	L 231	Bristles.
11. Scute—2	sc_2	Feb., 1920	W 945	Bristles.
12. Scute—3	sc_3	May, 1920	W 1346	Bristles; fertility of ♂♂.
13. Yellow	y	Oct., 1921	R 2	Color of body, wings, etc.
14. Peach	p	May, 1920	W 1384	Eye color.
15. Beaded	be	Nov., 1920	W 1964	Wings; viability.
16. Rough	r	March, 1919	L 9	Texture of eye.
17. Triple	tr	{ Dec., 1919 May, 1920	W 754 W 1498	Wings.
18. Deformed	d	Nov., 1919	W 360	Almost every part of body.
19. Serrate	st	March, 1920	W 1146	Texture and size of eyes.
20. Rimmed	ri	May, 1919	W 1	Wings and scutellum; viability.
21. Pale	pa	Feb., 1920	W 980	Bristles; viability.
22. Stump	s	June, 1919	L 254	Wing vein.
23. Short	sh	Feb., 1920	W 1110	Wing veins.
24. Short—2	sh_2	May, 1920	W 1440	Wing veins.
25. Short—3	sh_3	March, 1920	W 1164	Wing veins.
26. Morula	m		L 411	Texture of eyes; viability.
27. Nicked	nk	June, 1919	W 11	Wings.
28. Rosette	ro	Nov., 1919	L 438	Almost every part of body.

In Tables II–VI parentheses indicate data omitted from the regional summary on account of poor viability of one class or else inability to classify one class. The two columns under the respective headings represent complementary classes, the one to the left that includ-

ing the normal allelomorph of the gene farthest to the left. *E.g.*, in Table II, experiment 3, under crossovers in region 1 there are 48 normal bristle rough eye flies, and 55 stubby bristle normal eye flies.

The plus sign (+) indicates wild-type or normal.

TABLE II

Two-point Crosses

Experiment Number	Nature of Cross	Non-crossovers		Crossovers in Region 1		Total
3	sy r × +	71	73	48	55	247
4	o sb × +	1,031	796	34	28	1,886
5	o bn × +	285	275	36	25	621
20	f × ri	66	70	26	20	182
29	sc × ri	304	291	31	29	655
35	p r × +	131	81	5	7	224
36	p s × +	100	111	20	21	252
38	r ri × +	127	73	1	1	202
39	r × s	173	170	29	26	398
40	s sh_3 × +	495	(560)	37	(0)	532
41	s × sh	126	(169)	14	(0)	140

TABLE III

Three-point Crosses

Experiment Number	Nature of Cross	Non-crossovers		Crossovers in Region 1		2		1, 2		Total
2	sy sb × bn	704	582	28	29	40	38	0	0	1421
6	o bn × f_2	35	66	6	8	16	8	3	0	142
18	f r × re	243	238	40	35	12	25	0	0	593
19	f s × sc	48	50	10	9	14	14	1	0	146
23	sc ri × r	230	189	20	17	3	3	0	0	462
26	sc r s × +	510	460	26	28	55	45	4	2	1130
28	sc s × d	258	324	23	25	25	34	1	1	691
32	sc ri × m	198	265	42	28	60	26	0	6	625
33	sc s sh_3 × +	174	(146)	(54)	55	(8)	19	3	(0)	251
34	p r × be	(20)	168	3	(0)	(1)	5	0	0	176
37	r ri × tr	312	281	2	0	1	5	0	0	601

TABLE IV

FOUR-POINT CROSSES

Experiment Number	Nature of Cross	Non-crossovers	Crossovers in Region							Total
			1	2	3	1, 2	1, 3	2, 3	1, 2, 3	
1.	sy r × o sb	321 325	9 4	22 12	244 205	0 0	1 1	7 4	0 0	1,156
7.	o re × f s	40 50	15 20	3 13	15 13	1 2	6 5	1 3	0 0	187
9.	o f r × ri	161 150	70 88	58 51	10 2	8 10	2 4	2 1	0 1	618
10.	o r s × t	48 57	30 27	15 23	7 15	5 1	3 3	1 1	2 2	240
12.	o r s × tr	37 56	40 50	0 1	6 7	0 0	8 2	0 0	0 1?	208
13.	o d × r ri	12 19	6 7	0 2	0 2	0 0	0 0	0 0	0 0	48
15.	f re sc_2 × r	290 311	54 43	9 4	43 26	0 0	1 0	0 0	0 0	781
21.	re sc_2 × be r	(89) 268	1 (0)	4 (0)	(3) 6	0 0	0 0	0 0	0 0	279
22.	sc r s × d	220 160	16 20	8 0	14 13	0 1	1 1	1 0	0 0	455
24.	sc ri × r s	348 306	41 18	6 12	25 17	0 1	3 2	0 0	0 0	779
27.	sc r s × nk	250 306	31 23	42 27	28 10	1 4	3 9	1 19	0 0	754
30.	sc ri × pa m	(38) 108	10 (2)	7 (0)	(6) 7	0 0	0 (1)	0 0	0 0	132
31.	sc ri m × s	149 43	2 15	12 6	12 13	2 2	1 0	0 0	0 0	257

TABLE V

FIVE-POINT CROSSES

Experiment Number	Nature of Cross	Non-crossovers	Crossovers in Region											Total
			1	2	3	4	1, 2	1, 3	1, 4	2, 3	2, 4	3, 4	1, 3, 4	
8...	o f × sc_3 ri s	64 58	27 31	9 9	11 2	6 2	2 2	1 3	3 2	1 0	1 2	1 2	0 0	239
16...	f re × sc r s	167 204	20 18	3 3	8 6	17 28	0 0	0 1	2 1	1 0	1 0	0 0	0 0	480
17...	f re sc_2 r × ro	232 222	41 27	1 1	21 14	190 75	0 0	0 0	6 21	0 0	1 0	2 11	1 0	866
25...	sc ri × r s nk	122 140	10 7	2 0	11 0	27 6	0 0	0 2	0 5	0 0	0 3	0 14	0 1	350

TABLE VI

SIX-POINT CROSSES

Experiment Number	Nature of Cross	Non-crossovers	Crossovers in Region																Total
			1	2	3	4	5	1,2	1,3	1,4	1,5	2,4	2,5	3,4	3,5	4,5	1, 2, 3	1, 2, 5	
11.	o sq r s × sc ri	92 60	41 50	19 4	10 4	2 1	9 9	3 3	2 3	1 2	1 6	0 1	1 1	0 1	2 0	0 0	0 1	0 2	331
14.	f re sc_2 r × p s	180 152	16 18	1 1	3 3	11 8	22 18	0 0	0 0	0 1	2 3	0 0	0 0	0 0	0 2	1 0	0 0	0 0	442

TABLE VII

SUMMARY OF ALL AVAILABLE DATA BETWEEN CONSECUTIVE LOCI

Region	Cross-over Value	Number of Flies	Region	Cross-over Value	Number of Flies	Region	Cross-over Value	Number of Flies
sy–o[6]	1.3	1,156	t–r[6]	20.8	240	r–a[6]	2.4	503
sy–sb	4.0	1,421	sq–sc[6]	10.6	331	r–ri[6]	2.3	2,742
sy–r	41.7	247	re–sc[6]	0.95	2,848	r–s[6]	11.1	3,444
o–sb[6]	3.5	3,042	re–r	6.2	593	r–ro[6]	35.4	866
o–bn	10.2	763	re–s	23.0	187	tr–ri[6]	1.0	601
o–f[6]	29.0	1,044	sc_2–p[6]	1.8	442	tr–s	11.5	208
o–t	30.4	240	sc_2–be	1.4	279	d–ri[6]	4.2	48
o–sq	34.7	331	sc–r[6]	7.1	6,388	d–s	7.9	1,146
o–r	44.5	256	sc–d[6]	7.2	691	ri–pa[6]	5.3	132
sb–bn[6]	5.5	1,421	sc–ri	9.9	1,908	ri–s	7.5	1,956
sb–r	40.0	1,156	sc–s	21.9	397	ri–m	14.7	625
bn–f_2	19.0	142	p–be[6]	1.7	176	pa–m	5.3	132
f–re[6]	11.2	3,349	p–r	5.0	654	s–sh[6]	7.9	923
f–sc[6]	11.9	385	p–s	16.3	252	s–m[6]	10.1	257
f–r	21.2	618	be–r	2.4	455	s–nk[6]	11.5	1,100
f–ri	25.3	182	r–tr[6]	0.5	809			

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[6] These data were used in the construction of the chromosome "map."

INHERITANCE OF PLUMAGE COLOR IN CROSSES OF BUFF AND COLUMBIAN FOWLS

DR. L. C. DUNN[1]

As a part of a search for material suitable for use in measuring the linkage strength of several sex-linked characters in poultry, some preliminary experiments have been undertaken on the inheritance of the Columbian plumage pattern. The results of these experiments have confirmed those of Sturtevant (1912) in establishing the sex-linked nature of one of the genes involved in the production of this pattern, and have demonstrated the relationship between it and the buff plumage coloration. The inheritance and somatic effects of the chief factor involved appear to be clear enough to make it useful in genetic investigations on poultry. A short description of the experimental results is therefore given here, to be followed by a more detailed report when further evidence is at hand.

The Columbian pattern, sometimes known as the Ermine coloration, is characteristic of several standard varieties of a number of breeds of poultry of which the Light Brahma, the Columbian Plymouth Rock and the Columbian Wyandotte are perhaps the most familiar. Although subject to some variation the pattern consists in general of a pure white surface color in all parts of the plumage except in the hackles, wings, and tail feathers, in which black is present either as a central stripe (hackles); as a solid color covering somewhat more than half the feather (primaries) or as a solid color covering the whole feather (tail). In typical Columbian fowls the undercolor or fluff at the base of the body feathers is generally lead or slate, which is sometimes so pronounced as to show through at the surface especially on the back.

[1] Contributions in *Poultry Genetics*, Storrs Agr. Exp. Station.

This pattern is alike in both sexes except for the slightly different appearance caused by structural differences in the hackle and saddle feathers.

The down color of newly hatched Columbian chicks is white or a yellowish white like the down characteristic of the chicks of many white varieties of poultry, *e.g.*, White Leghorns. Black or gray markings appear on most Columbian chicks as a spot on the head, or as dorsal stripes on the head or back and in the developing wing quills. This pattern varies in different individuals from an entire absence of dark pigment to the presence of rather heavy dark dorsal stripes.

The color variety chosen for crossing with Columbian was buff, since this offered a clear contrast in the absence of white and the uniformity of coloration and because the plumage color of both sexes is practically the same. Moreover, buff is known to be recessive to many other plumage colors and patterns and for this reason is less likely to carry other factors which might complicate the results.

The first crosses were made between a Columbian male extracted from the second generation of a cross between Light Brahma and White Leghorn,[2] and purebred Buff Orpington females.

Twenty-three chicks were hatched from these crosses. Of these, twelve were predominantly white in the down, and eleven were buff. Of the whites four were pure white, five had a black streak or spot on the head and saddle, and black pin feathers appearing in the wings, and closely resembled purebred Light Brahma chicks in color; one was smoky white and two were white with a buff spot or streak on the head and neck. Of the buffs eight were clear buff in color, one was a very light buff

[2] The cross of Light Brahma by White Leghorn (quoted by permission from unpublished data of Sinnott and Warner) when made reciprocally produced white birds in F_1, generally with some ticking with black and occasional brassiness or tinging with buff. The White Leghorns used were apparently pure for the dominant inhibitor of color (I) while the Light Brahmas contained the recessive allelomorph of this gene (i).

and two were buff with black down on heads and saddles and with black feathers in the wings. All of the white chicks developed adult plumage resembling the Columbian pattern except that the black in the hackles, tail and wings was a dingy gray, occurring as stippling on the white ground rather than as a solid color. The buff chicks which survived developed adult plumage in which the hackles, tail and wing feathers were gray or black, while the feathers over the rest of the body were buff. These resembled mosaics of buff and Columbian in which the Columbian pattern was imposed on a buff ground.[3]

In tabular form the results of this cross were as follows:

TABLE I

Columbian Male × Buff Female

	White		Buff	
Down Colors	12		11	
	♂	♀	♂	♀
Adult Colors	6	6	3	6

The appearance of two kinds of offspring in equal numbers from this cross indicated that one parent was probably heterozygous in a factor causing the difference between white and buff. Later work showed this to be the male. When a *purebred* Light Brahma male was mated to purebred buff females (Orpingtons and Plymouth Rocks) the thirty-seven offspring were without exception white in the down and developed into white Columbians as adults. The dominance of white over buff was practically complete although one or two buff feathers were noted on one hybrid and a slight buff tinge on another.

[3] The resemblance of these hybrids to descriptions and illustrations of early buff varieties (Tegetmeier, 1872) is quite striking. At present the only buff variety characterized by a considerable amount of black in hackles, wings and tail is the Buff Brahma, although this is not yet recognized by poultrymen as a standard type.

The coloration characteristic of the Rhode Island Red breed is essentially an ermine or Columbian pattern with a red ground substituted for the white of true Columbians. A very useful discussion of the relationships between these patterns from the standpoint of a breeder and fancier of long experience is given by Robinson (1921) see esp. pp. 55 and 56.

The offspring of the purebred Light Brahma male by buff females were much whiter than the chicks from the first male. Only one of the thirty-seven showed the dark head spot characteristic of Light Brahma chicks and all were of a clear dead white, lacking even the yellowish tinge characteristic of most white chicks in the down. As adults these birds were very similar to the first lot. The amount of black in hackles, tails and wings was about intermediate between the amount present in the Columbian parent and the absence of black in pure white birds.

The first generation hybrid chicks were crossed in two ways. The F_1 Columbian females were backcrossed to a purebred Buff Plymouth Rock male; the F_1 buffs were bred *inter-se*. The results of these matings are presented in Tables II and III.

TABLE II

RESULT OF CROSSING F_1 COLUMBIAN FEMALES WITH PURE BUFF MALE

	White		Buff		Total
Down Colors	36		38		74
	Columbian		Buff		
	♂	♀	♂	♀	
Adult Colors	14	0	0	21	35[4]

TABLE III

RESULT OF CROSSING F_1 BUFFS INTER-SE

	Buff and White		Buff		Total
Down Colors	9		74		83
	Columbian		Buff		
	♂	♀	♂	♀	
Adult Colors	0	0	17	15	35[4]

From the backcross of F_1 Columbian females with a buff male equal numbers of buff and white chicks resulted, a clear monohybrid segregation. Evidently one factor determines the difference between white and buff, and from the F_1 results it is clear that white is the dominant allelomorph. This factor is however sex-linked, since

[4] The differences between the numbers of chicks and the numbers of adults indicate the number of birds which died before definitive plumage or secondary sex characters were developed.

all sons of the F_1 Columbian females are white (Columbian) while all the daughters are buff.

The mating of F_1 buffs *inter-se* produced only buff chicks, indicating that buff is recessive and breeds true. The nine chicks recorded as buff and white all had buff heads or wings or both and those which lived developed buff adult plumage. Genetically they were probably extremely light buffs.

As regards only the difference between white and buff, we may conclude that the Columbians contain a dominant sex-linked gene for the inhibition or restriction of buff from the plumage. The first male was evidently heterozygous for this factor; the second male was homozygous for it; the Columbian females contained but one dose of it, and this was located in the single sex chromosome; while all the buffs lacked it entirely. This is evidently the same gene (I) which Sturtevant (1912) found in Columbian Wyandottes, although its effects were somewhat obscured by other factors in his crosses with Brown Leghorn.

The presence of this gene in some White Wyandottes which I have studied strengthens the homology between the gene with which Sturtevant was dealing and the gene which is present in the Light Brahmas used in these experiments. I have recently crossed two White Wyandotte males with purebred Buff (Orpington) females. The white males were known to be recessive white (cc), *i.e.*, they lacked the gene (C) for the development of color in the plumage. The results of this cross are shown in the table following.

Result of Crossing White Wyandotte Males with Buff Orpington Females

	White			Buff		Total
Down Colors	13			13		26
	Columbian			Buff		
	♂	♀	[illegible]	♂	♀	
Adult Colors	3	2	1	3	7	16

In addition to the types noted above three unclassified

chicks were born from one mating. These were chiefly white in the down with black spots on the crown and neck and black quills in the wings. They resembled very dark Columbian chicks. These developed adult plumage different from the other chicks in this cross and could not be classified either as Columbians or buffs. Additional factors which have not been identified were probably contributed by one of the White Wyandotte males and further reference to these birds will therefore be postponed until more information is obtained. Omitting these, the salient fact concerning this cross is the production of only two classes of chicks, white (Columbian) and buff in equal numbers. The White Wyandotte males bred, therefore, like F_1 hybrids between Columbian and buff and were undoubtedly heterozygous in the gene for the restriction of buff. As adults the offspring of this cross were indistinguishable in color from the offspring of the heterozygous Light Brahma male first used in crosses with buffs. The amount of black in the hackles of these birds appeared to be somewhat greater than in the offspring of the Light Brahma cross, but it was not sufficient to serve as a distinguishing mark. White Wyandottes, therefore, may carry a gene for the restriction of buff which is probably the same as the gene found in Light Brahmas and Columbian Wyandottes.[5] It is not demonstrable, of course, except in crosses of White Wyandottes with colored fowls which supply the dominant gene *C* for the development of pigment.

In addition to these three instances of the occurrence of a gene for restriction of buff there are numerous other cases in the literature in which the difference between buff (or red) and white in certain parts of the plumage is apparently due to the same gene or one with similar effects. Davenport (1912) found a sex-linked dif-

[5] Professor W. A. Lippincott has called my attention to this statement in Robinson (1921), p. 42: "The pattern (*i.e.*, Columbian) was also produced by crossing the Rhode Island Red (which has really the same pattern with the black—on a red ground—reduced to a minimum) with a White Wyandotte."

ference between Dark Brahmas and Brown Leghorns, the gene "W" inhibiting the appearance of buff or red in the hackles and saddles of Dark Brahmas. Jones (1914) was probably dealing with a similar sex-linked gene in his crosses between Silver and Golden Campines, and Hagedoorn's (1914) evidence indicates that the same or a similar sex-linked gene differentiates Silver and Golden Assendelvers. Punnett (1919) distinguishes a sex-linked gene "S," which in crosses of Silver and Golden Campines inhibited the development of buff or gold in the plumage, leaving certain portions of the feather "silver" or white. Most recently evidence presented by Haldane (1921) indicates that black and white barring such as characterizes the Barred Plymouth Rock variety is differentiated from black and buff (or red or gold) barring by the same gene "S" for the inhibition of buff. This sex-linked gene "S," Haldane found to be linked, as was to be expected, with the sex-linked gene "B" (barring).

In each of these cases a dominant sex-linked gene was found which restricted or inhibited the development of buff (or red or gold) in certain parts of the plumage. Although in the absence of data on crosses between the varieties mentioned it is impossible to assert that the restriction of buff in the silver or white-patterned varieties is in each case due to the same gene, the presumptive evidence in favor of such a view is strong. It appears probable that Columbian and buff varieties of several breeds (Leghorns, Plymouth Rocks, Wyandottes, etc.) are differentiated by the presence in the Columbians of a gene for the inhibition or restriction of buff pigment; and in view of the history of the various color varieties that this gene has been introduced into and now differentiates Golden from Silver-laced Wyandottes, Golden from Silver Spangled Hamburgs, gold pencilled (or partridge) varieties from silver-pencilled ones and other golden varieties from silver varieties which differ only in the distinction between buff and white in the plumage.

Data on the results of crosses involving these color varieties are urgently needed, and the generalization offered above is put forward as a temporary simplification in lieu of but as an aid to more extensive research.

The Black Component of the Columbian Pattern

When the experiments with buff and Columbian fowls were begun it was supposed that at least two alternative characters distinguished these varieties; viz., ground color (white as opposed to buff) and pattern (black in hackles, wing, and tail as opposed to self coloration). The results of these experiments, a reexamination of the parent types and a cursory review of poultry literature indicate the error of this assumption.

1. *Experimental.*—The first generation of the cross Columbian × Buff consisted of birds intermediate between the parental types in the amount of black pigment present. If four arbitrary grades (1–2–3–4) in the reduction of the amount of black in hackles, wing and tail are made between typical Columbian and entire absence of black (white or buff self) then the first generation is found to consist of the following grades.

Columbian	—1	—2	—3	—4	Self
0	8	10	2	0	0

If the buff parents are classified as self then the hybrids resemble the Columbian parent more closely. But a careful examination of all the buff females used revealed the presence in each of them of a small amount of black pigment usually as broken patches or fine stippling in the tail and primary feathers, and occasional traces in the hackles.[6] The buffs, therefore, can not be regarded as selfs and most of those used in these experiments were assignable to grade–4. The amount of

[6] It is the experience of farmers and poultrymen, as evidenced in poultry literature, that buff fowls with no admixture of black pigment have been rare. Black in wings and tail is being rigidly selected against and is being gradually reduced in modern breeds.

black in the F_1 fowls is only slightly above the mid point between Columbian and grade–4.

An F_2 generation has not yet been raised from the cross of typical Columbian × Buff, but some data are available on the F_2 generation from the cross of the heterozygous Light Brahma which was first used in crosses with buff. This male was lighter than typical Columbian, about grade – 1. His offspring were not graded but had slightly less black than the offspring of the typical Columbian male, averaging about grade –3. The amount of black was similar in the Columbian and buff progeny. When these F_1 buffs were bred *inter-se* the grades of the F_2 adult fowls were as follows:

Columbian	—1	—2	—3	—4	Self
0	7	13	10	1	0

The variation in amount of black pigment was practically continuous, except that the Columbian parental type was not recovered. No buffs were obtained which were entirely free from black in tails or wings.

The F_1 Columbians were backcrossed with a pure Buff Rock male which showed only faint traces of black stippling (mealiness) in the tail. The progeny of this cross were of the following grades:

Whites (Columbians) and buffs combined:

Columbian	—1	—2	—3	—4	Self
0	2	3	9	13	4

The amount of black in these fowls was obviously much less than in the F_2 generation although the same grades were represented. In four fowls (three buffs and one white) no trace of black pigment could be detected.

It is obvious from these facts that as regards the black component of the Columbian pattern, the Light Brahmas and the buffs used differ only in amount. A blend occurs in the first generation followed by segregation in the second and backcross generation. It is probable, there-

fore, that the two types crossed differ from each other by multiple factors affecting the amount of black pigment produced. The number of these factors can not be estimated because of the small numbers of animals involved and because a second generation has been bred only from an original cross in which the Columbian parent did not have the amount of black normal to that variety. Failure to recover the typical Columbian pattern in later generations is probably due to the last named circumstance rather than the absence of segregation.

The two varieties probably do not differ by any single factor determining the presence or absence of black pigment, but only in the degree to which black is produced, the degree probably being governed by accessory or modifying factors. This fact attaches especial interest to the appearance of several birds in the backcross generation which show no trace of black pigment. Do these represent loss of a factor determining the ability to develop any black pigment at all or are they segregates in which factors limiting the exercise of the black-producing function are at a maximum? Since they are few in number and since variation in the amount of black grades imperceptibly into the self condition I am inclined to the latter view. If this is true it should be possible to reduce the amount of black in Columbian fowls by rigid selection against it to such a point that birds might be produced which were phenotypically white, but which as regards restriction of buff would breed like Columbians.[7] Such a character would be in effect a sex-linked self white and the absence of a sex-linked white in the many breeds investigated points to the probability that none has been produced in this way.

Much of the interest in the case presented here inheres in the apparent simplicity of the results. The crossing of Light Brahmas and Buff Orpingtons or Buff Plymouth Rocks produces in the first, second and backcross generations only two easily distinguishable types, white (Co-

[7] One such bird has appeared in the course of these experiments; see p. 244.

lumbian) fowls and buffs—in which to be sure there is some variation in the amount of black pigment present in certain parts of the body, but apparently no epistatic pattern factors are introduced from either side of the cross to obscure the visible segregation of the main factors. Restriction of buff as found in Columbian fowls is, therefore, a valuable sex-linked gene for use in measuring linkage or in other Mendelian experiments with poultry while buff appears to be the best color variety to be used in studying the inheritance of unknown plumage characters. The Brown Leghorn or game type plumage pattern, although it resembles the supposed wild type form, is in the writer's opinion less valuable than buff because of the often evidenced[8] presence in the genetic constitution of the Brown Leghorn of epistatic pattern factors for extension of black pigment, stippling, etc.

The general results of the experiments reported have been to confirm and extend the previously known facts regarding the inheritance of the Columbian variation. The genetic relationships of this pattern and the buff coloration also throw some interesting light on the evolution of these two color varieties. They differ, it has been shown, only in one main gene which determines the production or restriction of buff in the plumage. Both are able to develop black pigment in certain parts of the plumage, while they differ quantitatively in the degree to which black may be produced. The former single factor difference probably arose as a single mutation, while the latter and less important difference is one which could be brought about by selection of small variations which had already arisen in a common parental stock.

The variation which produced the chief difference between these two color varieties, *i.e.*, the restriction of buff, undoubtedly took place at least 75 years ago and probably in China, although there is no evidence that the same variation has not occurred several times. The first known Columbian breed was probably the Gray Shang-

[8] Sturtevant, A. H. (1912); Lefevre, G. (1916).

hae, from which the Light Brahmas were derived. These fowls were imported into the United States from China in the decade before 1850[9] and into England shortly afterward. At about the same period and often in the same shipments were imported certain buff birds which eventually became the foundation stock of the Buff Cochin breed from which practically all buff varieties of the present day received their color. These two varieties were practically identical in characters other than plumage color[10] and in the matter of plumage the chief difference was the difference in body feathers, being white more or less stippled with gray in the Shanghaes and buff similarly stippled with gray (mealiness) in the Buff Cochins. In China, observers have regarded the buff as the older color variety while the gray was noted as separate about 1840.[11] The Chinese apparently paid little attention to color in breeding their fowls and the variation from buff to white (or the reverse) in the plumage may have occurred many years or even centuries previous to this date.

The further differences between Columbian and Buff breeds have taken place since their introduction from the Orient, chiefly under the selective breeding of English and American poultrymen. The buffs were at first characterized by a great deal of variation in the shade of the principal color—ranging from lemon to red; while the wings, and tails, and tips or margins of the hackles varied from solid black through stippling and blotching to an absence of black in any one of these parts.[12] All subsequent selection has been against the black[13] and the American Standard of Perfection now specifies "buff in all parts of the plumage." In the Shanghaes or Light Brahmas on the other hand the object of the breeder

[9] Weir, Johnson and Brown, "The Poultry Book," N. Y., 1912, p. 528.

[10] Tegetmeier, W. B., *loc. cit.*, p. 63.

[11] Weir, Johnson and Brown, *loc. cit.*, p. 528.

[12] Weir, Johnson and Brown, *loc. cit.*, p. 527; p. 630, p. 540. Tegetmeier, *loc. cit.*, pp. 40–41.

[13] With the exception of the selection for black in hackles, wing and tail which was employed in developing the Buff Brahma variety.

has been to preserve the black in the hackles, wings and tails and to heighten the contrast with the white body by selecting against grayness or mealiness in the body feathers. Two principal processes were apparently involved in the production of buff and Columbian varieties; a discontinuous change or mutation producing the chief difference, and the accumulation by selection of minor factors producing the minor changes. It is impossible to say whether the buff and Columbian varieties which exist at the present time in the principal breeds were derived from these original types by crossing or whether the principal mutation and the minor changes and selection have recurred in the different breeds. The probabilities are in favor of the first alternative.

SUMMARY

1. The Columbian plumage coloration in domestic fowls is distinguished from buff coloration by the presence of a gene *S* which determines the restriction or inhibition of buff pigments from the feathers. This gene is sex-linked, and dominant over its allelomorph *s*, which permits the development of buff pigment.

2. Fowls with the Columbian coloration do not differ from buff fowls in any single gene governing the development of black pigment. Multiple genes appear to determine the difference in the amount of black pigment developed.

3. Columbian and buff fowls are genetically alike in plumage pattern, that is, in the ability to develop black pigment in the feathers of certain areas (hackle, wing and tail feathers).

4. The buff coloration appears to have diverged from the Columbian coloration, or the reverse, by a single gene mutation affecting the development or inhibition of buff pigment; and by the accumulation through artificial selection of multiple genes for the development of black pigment in the Columbian varieties of fowls, and by the reverse selection in most buff varieties.

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FURTHER NOTES ON THE PALEONTOLOGY OF ARRESTED EVOLUTION

DR. RUDOLF RUEDEMANN

STATE MUSEUM, ALBANY, N. Y.

THE writer has in a former paper[1] endeavored to follow up the causes of persistence as seen from the side of the paleontologist. Using as a basis the genera which appear in Zittel-Eastman's Textbook of Paleontology (1913) and defining as persistent all genera which pass through more than two periods, the following data relative to number of persistent genera (*A*), total number of genera cited (*B*), and percentage of persistent genera (*C*) were obtained:

	A	*B*	*C*
Foraminifera	48	86	56
Sponges	9	149	6
Corals	46	237	15
Echinodermata:			
Crinoidea	5	277	2
Cystoidea	0	96	0
Blastoidea	1	23	4
Ophiuroidea	0	25	0
Asteroidea	5	43	11
Echinoidea	19	191	10
Bryozoa	68	306	22
Brachiopoda	33	384	9
Mollusca:			
Pelecypoda	78	446	16
Scaphopoda	5	18	27
Gastropoda	126	420	30
Pteropoda (so-called)	5	17	29
Pulmonata	7	65	11
Cephalopoda (*a*) Nautiloidea	12	170	7
(*b*) Ammonoidea	0	455	0
(*c*) Dibranchiata	0	...	0
Crustacea			
Trilobita	6	131	4.5
Ostracoda	18	68	26.5
Cirripedia	4	20	20
Malacostraca	7	134	4.5
Arachnida	3	66	4.5
Selachii	16	168	9.5 (in first edition)

[1] R. Ruedemann, "The Paleontology of Arrested Evolution." Presidential Address. Albany, 1916. New York State Museum Bull. 196, 1918, pp. 107–138.

Dipnoi, Teleostei, Reptilia each have one in the 1896 edition of Zittel-Eastman. The vertebrate volume had not yet appeared of the second edition.

From an analysis of the percentages we drew the following inferences:

1. The lower classes tend in general to have more persistent types than the higher.

2. Within each order and class, again, the lower subclasses tend to furnish the greater percentage of persistent forms.

3. Frequently the persistent genera form a primitive central stock from which numerous shorter lived genera branch off.

4. The stable conditions of the open ocean and deep sea (as in the Foraminifera) and the subterranean conditions favor persistence of types, the latter condition including the burying and boring forms.

5. Sessile forms contain more persistent types than the vagile benthos.

6. Persistent types prevail in much greater number among the marine forms than among the land and freshwater animals. Among the continental forms again the limnal and fluviatile forms appear to be more persistent than the terrestrial forms.

7. Most persistent types are small and inconspicuous forms.

8. Many persistent genera show a slow development, a distinct climacteric period and a long post-climacteric period. Connected with this observation is the other that persistent genera which slowly develop never produce many species during a single geologic period.

9. Minor factors of persistence are seen in (*a*) extreme individual vitality (as in *Lingula* and *Crania*), (*b*) immense broods (as in *Ostrea* and *Limulus*), (*c*) extreme restriction in the matter of food, as in the eaters of carrion and refuse (Capulidæ, oyster, etc.).

The same criteria were found to hold, on the whole, in regard to the persistent species and the higher groups

(families and orders). In the latter case superior sets of offensive arms and defensive armors, early developed, appear to have helped to give stability to some, as in the scorpions (pincers and poison glands), limulids (leathery armor combined with burrowing habit and enormous broods). Some, as the turtles, have successfully specialized for protection.

In trying to reduce the multiplicity of factors to a few controlling agents, it was found "that these are the fixation of the 'over-taken' and post-climacteric types, the presence of stable physical conditions, and withdrawal in various ways from the fields where the struggle for existence is fiercest. The stable physical conditions have been found by many in the open ocean, by some in the deeper littoral regions of the oceans, by others again in subterranean fields, by some in the rivers and lakes of continental regions that remained undisturbed by folding. Withdrawal from the struggle for existence with other organisms has been accomplished by a variety of means, as by isolation, burrowing life, small, inconspicuous size, superior, often deadly, offensive and strong defensive arms, through restriction to poor fare, great power of endurance, etc."

In an analysis of the biologic factors that have permitted persistence, two entirely different groups of persistent types must be distinguished: (1) The post-climacteric types; (2) the primitive central stocks. The former rely on stable physical conditions and withdrawal from the arena of the struggle for existence, as far as possible; while the latter are frequently dominant in the very seats of war. We have termed the first *persistent terminals,* the others *persistent radicles.*

The persistent terminals were considered to have become so fixed in all their characters as to make them persistent partly by the factors of progressive fixation and partly by the fact that they have in various ways avoided the opposing factor of natural selection; their conservation thus being in fact due in part to their ge-

rontic condition and in part to the peacefulness of their surroundings.

The persistent radicles, on the other hand, were thought to owe their persistence to the fact that through their primitive nature they are still adapted to a greater variety of conditions and that while there may be considerable variation, it is around a still unspecialized, primitive form and thus difficult of recognition.

Or, expressing the same difference in terms of the four processes of heredity, ontogeny, environment and selection, around which, according to Osborn, the life and evolution of organisms continuously center, we found that "the difference between the two groups of persistent types, the relatively rigid terminals and the more variable radicles, consists in the fact that in the former all factors have become fixed and unresponsive to stimuli, only the selection still slowly acting, while the latter are so well adapted to a variety of conditions that no changes readily originate through any of the processes of environment, ontogeny and selection, which affect the whole stock, while at the same time no changes in the germ plasm are induced through hereditary tendencies."

The following notes are written with the intention partly to add certain new factors that appear to contribute to the persistence of forms, and that had not been taken into account in the first essay; and partly to enter deeper into the analysis of the ultimate causes of persistence made possible through more recent investigations into the nature of phylogenesis.

1. Additional Factors of Persistence

The new factors here mentioned have all to do with the methods of reproduction whose influence had not been recognized, in the first paper, in the percentage table of persistent genera.

(*a*) *Reproduction by Simple Division.*—In the Protozoa reproduction takes place by division without any

loss, so that there is no distinction between parent and offspring. There is no death and thus it is that Weismann and others have spoken of the "immortality of the Protozoa." It is certainly significant, in this connection, that among the Foraminifera 56 per cent. of the genera were found to be persistent and many were found to exhibit tremendous persistence, ranging from the Ordovician, Silurian, Carboniferous and Triassic to recent times, and that even species (see Ruedemann, *op. cit.* p. 126) are known to extend from Silurian, Devonian, Carboniferous and Triassic times to the present. These forms the writer designated as actual "immortal types" in contrast to the theoretically immortal protozoans of Weismann.

There occurs, however, among the protozoans besides this asexual mode of reproduction a group of processes that are clearly the primitive beginnings of fertilization. In these forms of conjugation different stages may be distinguished, viz., the mere congregation of cells in groups without visible exchange of plasms (cytotropy); the exchange of substance taking place only through osmotic processes; further conjugation, where real fusion of plasmas occurs but the cell-nuclei remain separate (plastogamy); and finally such modes of conjugation, where also nuclear fusion of the conjugating cells takes place (karyogamy); and here again, the pairing cells may be either similar in size (isogamy), or even markedly dissimilar in size (anisogamy).

It is, however, to be remembered that the usual reproductive process among protozoans is simple fusion of ordinary vegetative cells and conjugation as a rule occurs at rare intervals in most forms, often only when unfavorable conditions arise, or as Maupas' experiments indicate, the individuals in the course of numerous successive asexual generations grow old.

(*b*) *Reproduction by Budding.*—This mode of asexual reproduction differs from that of division originally in the protozoans merely in the different sizes of the daughter-

cells and the mother-cell, but develops into a complex process in the multicellular forms. Distinct budding occurs already in the protozoans as in *Arcella,* where a number of small buds are constricted off all round. In sponges it is developed to such a degree that no one can fail to recognize the impossibility of drawing any rigid line between growth and asexual reproduction.[2] In the cœlenterates asexual reproduction runs riot, as Geddes and Thompson state. It is, further, by far the prevailing mode of reproduction among the stock-building bryozoans; it also is common among marine worms, as with the famous palolo-worm off the coast of Samoa, and finally it is also frequently found among the tunicates.

The primitive character of this mode of reproduction can not be doubted. It probably in all cases is an inherited character that persisted from the ancestral protozoans. It has by many zoologists been considered as an acquired character among the tunicates, but Van Name[3] has lately advanced good reasons for the conclusion that it is also a primitive character among the ascidians inherited from their remotest ancestors and that it is not a faculty that can be acquired secondarily.

Budding leads to the formation of colonies or stocks. These as a rule are not favorable to a swimming or vagrant mode of life, hence by far the majority of budding forms are sessile, although there are a considerable number of exceptions in the swimming siphonophores, ctenophores, floating graptolites, and compound swimming worms and ascidians. Since most of the colonial stocks are sessile, budding has often been considered as having been induced by a sessile mode of life and thus held to be a function that could be acquired. Its absence among the sessile cirripedes seems, however, to support Van

[2] Geddes, Patrick, and Thompson, J. Arthur, "The Evolution of Sex," London and New York, 1914, p. 205.

[3] Van Name, Willard G., "Budding in Compound Ascidians and other Invertebrates, and its bearing on the Question of the Early Ancestry of the Vertebrates," *Bull. Amer. Mus. Nat. Hist.*, Vol. 44. art. 15, 1921, pp. 275–282.

Name's contention that this function can not be acquired when once lost.

The fact that the sessile forms contain more persistent types (corals have 15 per cent., bryozoans 22 per cent.) than the vagile benthos would suggest that budding may be a mode of asexual reproduction favorable to the persistence of types; and that it may be the cause of the large percentage of persistent types among the sessile forms. It must here, however, be considered that also the sessile Cirripedia which lack the function of budding, have furnished 20 per cent. of persistent types; and further that in all the classes here considered budding is associated with sexual reproduction, often, as in many cœlenterates, in a regular alternation of generations. Moreover, the sexually reproducing brachiopods, gastropods and pelecypods have furnished large percentages of persistent types, a large number of which are sessile forms.

While thus budding would not seem to be the controlling factor in the persistence of the sessile forms, it is, nevertheless, true that budding may have a distinctly retarding effect upon the evolution of such forms, principally by the material decrease of the cases of sexual reproduction. As in the case of the corals, the number of new stocks that originate from sexual reproduction and finding a new lodging place, start new colonies, is very small when compared with the number of asexually produced individuals on the stocks. There are therefore many more generations of asexually than sexually produced individuals.

(*c*) *Reproduction by Hermaphrodites.*—Another factor that possibly may have contributed to the persistence of forms is hermaphroditism. Claus has pointed out that hermaphroditism finds most abundant expression in sluggish and fixed animals. "Among sponges, sea-anemones, corals, Polyzoa, bivalves, etc., we find frequent illustration of the association of fixedness and hermaphroditism" (Geddes and Thompson, *op. cit.*, p. 83). The origin of

hermaphroditism is still a matter of dispute (see Geddes and Thompson, pp. 83, 84) for while some, as Simon, attribute it to a plethora of nutrition (as especially in parasites), others are "content to interpret it as an adaptation to ensure fertilization, for the possibilities of pairing between separate sexes are certainly lessened if the animals are sluggish, sedentary or parasitic." There is likewise difference of opinion as to whether the stage of hermaphroditism is the lower, and the condition of distinct sexes has been derived from it (Gegenbaur), or whether it is a secondary condition, derived from primitive uni-sexuality as claimed by Pelseneer who considers it grafted on the female sex in Mollusca, Crustacea and Pisces (Geddes and Thompson, p. 84).

Considering its prevalence among the lowest classes with sexual reproduction, notably the sponges and corals, and again among the Cirripedia, we believe that hermaphroditism is in the former an inherited primitive character and in the latter an acquired one. At any rate, since it is so frequently and distinctly associated with sessility, as in the just mentioned Cirripedia, and in many pelecypods (oyster) and with sluggishness in other pelecypods and many gastropods, and since it is exactly these same groups which contain numerous persistent types, it seems probable that hermaphroditism is a further reproductive condition contributory to persistence.

(*d*) *Reproduction by Parthenogenesis.*—Parthenogenesis is the mode of propagation in at least one typically persistent genus, viz., *Apus;* but it has also become a confirmed physiological habit in other archaic types of crustaceans among the branchiopods, as notably in *Artemia,* the brine-shrimp, in *Branchipus,* and in *Limnadia;* further in the equally primitive water-fleas (*Daphnia* and *Moina*) and finally, among the ancient ostracods, also in some species of the common *Cypris.*

Of the whole class of Branchiopoda, which through paleontology, and notably through the recent amazing

discoveries of Walcott[4] in the Middle Cambrian of British Columbia, are proven to reach back to the oldest fossiliferous beds (in *Protocaris marshi* Walcott to the Lower Cambrian), *Apus* is the most remarkable and most often cited form in paleontologic literature. The writer has in a paper, now in press, shown that true *Apus*, identical in form of carapace and "shell glands" has been found in Permian beds of Oklahoma. It was before known from the Triassic Buntsandstein of the Vogesian Mountains. Its more than 70 pairs of gill-bearing feet and other primitive characters have made it the model of comparison for Paleozoic crustaceans, especially the trilobites. The Lower Cambrian *Protocaris marshi* is so closely allied to *Apus* that it was termed *Apus marshi* by Bernard. There is hence no doubt of the immense age of this type.

Apus is now so parthenogenetical in its reproduction that the males were not discovered until a hundred years after the description of the first and best known species (*A. cancriformis* Schäffer); and "von Siebold repeatedly investigated every member of a colony of *Apus*, once over 5,000 in number, without finding a single male. At other times he found one per cent. while in certain unknown conditions (probably when food is scarce and life generally unfavorable) the males may be developed in crowds" (Geddes and Thompson, p. 189). Similar conditions prevail in the brine-shrimp and the other branchiopods, cited above, as shown by Lereboullet and Nowikoff.

Parthenogenesis is associated with other strange habits in the three branchiopods, *Apus cancriformis*, *Limnadia hermanni*, and *Branchipus stagnalis*, which occur together in Europe. These creatures occur only after very wet seasons in puddles, road-ditches and other small pools, where their eggs have lain for decades in the dry mud, exposed to heat and frost. They develop with amazing

4 Walcott, Charles D., "Middle Cambrian Branchiopoda, Malacostraca, Trilobita, and Merostomata," Smithsonian Miscellaneous Collections, Vol. 57, No. 6, 1912.

rapidity, *Apus cancriformis* reaching in two weeks a full size up to five inches,[5] produce an enormous number of eggs and die.

The origin of parthenogenesis in these forms as well as in the rotifers and certain insects has been fully discussed by Geddes and Thompson, and they are certain that it has originated as a degeneration from the ordinary sexual process (*ibid*, p. 198) and is no direct persistence of a primitive ideal state. Their theory of parthenogenesis is that the ova that develop parthenogenetically " are to be regarded as incompletely differentiated female cells, which retain a measure of katabolic (relatively male) products, and thus do not need fertilization " (they form only one polar body). " Such a successful balance between anabolism and katabolism is indeed the ideal of all organic life. In parasitic fungi, sexual reproduction disappears, and surrounding waste products presumably help the purpose otherwise effected by sexual organs, so peculiarities in the conditions of parthenogenetic ova may explain the retention of the normal balance which makes division possible without the usual stimulus of fertilization. Abundant and at the same time stimulating nutrition (Rolph), early differentiation of the sex-cells (Simon), the general preponderance of reproductive over vegetative constitution (Hensen), their liberation before the anabolic bias has carried them too far, are among these favoring conditions."

Parthenogenesis thus appears as a degenerative asexual process arising from peculiar conditions, the most important of which appears to be temporary over-nutrition. As in the other asexual modes of propagation, in division and budding, the inference suggests itself readily that this suppression of fertilization must induce persistence, for as Geddes and Thompson point out (*ibid.*, p. 193) the establishment of parthenogenesis and the ab-

[5] See Bruno Weigand, "Mitteilung über das Auftreten der Limnadia Hermanni Ad. Brgt. bei Strassburg im September 1912," *Mitt. der Philomat. Gesellsch. in Elsass-Lothringen*, Bd. 4, Heft. 5, Jahrgang 1912; 1913, p. 730.

sence of fertilization probably involves some diminution in the frequency and range of variability and thus the establishment of parthenogenesis will be a handicap to evolution.

In the case of *Apus,* and its other associated branchiopods as well, it is probable that the successful adaptation to special conditions is a strong contributing factor in the establishment of persistence, as pointed out by the writer in the former paper. It is possible that *Apus* has existed under these conditions from very early times.

Summing up the evidence on persistence of types from the habits of reproduction, it seems that simple division, budding, hermaphroditism and parthenogenesis have each contributed to this persistence and in their way acted as factors that arrested evolution, and that thus help to explain the relatively large percentage of persistent types in the protozoans, sponges, corals, molluscs and the just mentioned branchiopods among the crustaceans.

While the facts thus seem to indicate that these modes of reproduction, other than the normal process of fertilization, were favorable to persistence in fossil types, it is, in the present stage of our knowledge of the meaning of fertilization, not so simple to recognize the underlying cause of their arresting influence on evolution.

The simplest explanation would obviously be to see in fertilization the principal cause of *variation*, as such authors as Treviranus, Brooks, Galton, Weismann and Oscar Hertwig have done. Weismann has insisted that the intermingling of two "germ-plasms" is an important fountain of congenital variation. It can be readily seen that, under this view, the retarding effect of fission, budding and parthenogenesis consists in the exclusion, or restriction to long intervals, of fertilization, thereby reducing variability and the possible action of selection. It is also plausible under this view that mutual fertilization between hermaphroditic individuals tends toward equalization of characters; and this tendency towards equalization is still more increased by fertilization within

the same colonial stock or neighboring colonial stocks of plantations. The most important of the disadvantages resulting from hermaphroditism would then be to reduce the variability which is necessary to progress in the struggle for existence.

While, however, the possibility is not denied that fertilization may be a controlling factor in variation, as stated, *e.g.*, by William E. Kellicott in his "Text-book of General Embryology," 1913, p. 216, it is also obvious, according to the same author, that the evidence for this view is still scanty and uncertain and, moreover, there are two exactly opposed views as to the nature of the relation. While Hertwig maintains that the effect of fertilization is to limit variation within a species, Weismann asserts that the effect of syngamy or "amphimixis" is to cause or promote variation.

Kellicott (*op. cit.*, p. 214) states:

> There is little direct factual evidence for or against these views, either one of which can be maintained upon theoretical grounds. In a few cases it is known that the amount of variability is not significantly different among sexually (gametically) or asexually (parthenogenetically) produced individuals of the same species. And from the standpoint of more recent studies upon heredity and variation the evidence is chiefly either negative or opposed to the idea that this relation constitutes an important element in the origin or present function of fertilization. The present aspects of this relation between fertilization and variation merge in the larger question of the relations with heredity.

While among the higher classes fertilization has become a stimulus to reproduction and a means of heredity, evidence from the lower groups tends to show that fertilization in its results has undergone evolution like every other organic function.

The view is widely accepted today (see Kellicott, p. 209) that among the Protozoa the processes of reproduction and fertilization are not fundamentally related, and the primary significance of fertilization must be sought in some other direction.

The observations made on protozoans have led to the

rejuvenation hypothesis, chiefly represented by Bütschli, Maupas and Richard Hertwig. "It has been found that protoplasmic activity tends gradually to diminish in intensity, and that associated with this diminution are certain morphological alterations in the structure and composition of the cell" (Kellicott, p. 209). These modifications are known as senescence, the senescent condition of the cell consisting frequently in the relatively large proportion of cytoplasm as compared with nuclear substance. Conjugation is assumed to restore the senescent protoplasm to its original condition of vigor, bringing about rejuvenation. It follows from this that protoplasmic activity is cyclic and that periods of senescence would lead to death unless fertilization should occur.

The real evidence for this cyclic character of the life processes of the Protozoans has been furnished by the observations of Maupas and Calkins on *Paramecium.* But observations of Jennings have shown that in different forms of *Paramecium* conjugation and rejuvenation may occur at very different intervals, and Woodruff has been able to prevent cyclic relations by substituting normal conditions for the artificial and more uniform ones of the laboratory. "By continually altering the character of the food, and by imitating in other ways the naturally variable conditions of pond life, he has been able to continue a single race of *Paramecium* for over five years" (quoting from Kellicott), during which period more than 3,000 generations were formed by simple fission. It follows from these observations that protoplasmic activity among the Ciliata may not be cyclic in character under certain conditions, and that when cyclic periods of depression or senescence do occur, the protoplasm may be restored to a condition of normal vigor. either by physical or chemical stimuli, or by fertilization (Kellicott, p. 212).

Fertilization is in these cases a form of reaction that takes place when external conditions become too uniform to bring forth the normal vegetative activities, and that

leads to an internal disturbance, thereby correcting the conditions of uniformity.

Applying these conclusions to our case of the persistent types it could be conceived that the reduction of fertilization to rare intervals, or its entire suppression, in the numerous persistent types that reproduce by fission, budding, parthenogenesis or hermaphroditism, produces a perpetual senescent condition that while not leading to death as in the rapidly dividing and sensitive Paramecium, finds its expression in the rigidity of the forms, recognizable in their lack of response to external stimuli and of further evolution, *i.e.,* in their persistence. Or in other words, infrequency or entire lack of rejuvenation through fertilization favors the persistent condition, at least among those persistent terminals that do not live under stable physical conditions. Those living under stable conditions may require fertilization as a necessary rejuvenating process counteracting progressive senescence and final extinction through lack of external stimuli. It would then appear that these lower modes of reproduction and very stable external conditions could not very well exist together.

However, as pointed out by Kellicott, there has been an evolution both of the process and of the consequences of fertilization, and the various possibilities as to the significance of fertilization are not mutually exclusive. It is therefore possible that the large percentage of persistent types among forms with more or less suppressed fertilization finds its explanation in some cases in the resulting lack of variation, in others in the resulting senescent and rigid condition of the race, and in still others it may be sought in the process of heredity, connected with fertilization. This last possibility will be dealt with in the following chapter.

Reduction of Factors to Fundamental Causes

The investigation of the various groups of persistent types has indicated that there are a variety of factors

involved in their production. Many of these were found to be connected with the environment, others, acting through variability, or its lack, with selection, and still others with the processes of heredity and ontogeny. While of these four fundamental processes of evolution, viz., heredity, ontogeny, environment and selection, that of selection may account for the cases of persistence where variability has been reduced to a minimum, possibly by the lower modes of propagation mentioned above, and that of environment accounts for persistence in those cases where the environment has become so stable as to lack the actual stimulus for further development, it is obvious that still more important factors are involved in heredity and ontogeny that make for persistence in organisms, especially as it is seen in the post-climacteric forms, or persistent terminals. Both the conservative process of heredity and the much less rigid one of ontogeny appear to become more or less fixed and inaccessible to changes in persistent types.

None of these four processes gives any clue to the actual mechanics of the factors that induce persistence. In trying to trace the latter to its ultimate causes, it becomes, therefore, necessary to go beyond these processes, and to appeal to the important conclusions that have been obtained by modern experimentation and observation regarding the methods of inheritance and production of new characters by means of the genes or character-determiners of the heredity-chromatin.

Among these conclusions especially suggestive in regard to our problem, are the views advanced by Dürken and Salfeld.[6] These authors have, one through an analysis of all recent zoological experiments on evolutionary problems, the other through a corresponding analysis of the evolution among the fossil ammonites, arrived at the view that variability or the appearance of new characters, and of new combinations of characters is produced in differ-

[6] Dürken, B., and Salfeld, H., "Die Phylogenese. Fragestellungen zu ihrer exakten Erforschung," Berlin, Gebr. Bornträger. 1921.

ent ways, by the genes; and not only through internal factors, as claimed by the Neo-Darwinian school, but also through external ones as demanded by the Neo-Lamarckians. The genes, which are not only actual units, or representatives of definite phenotypic characters, but definitely delimited, material bodies, may not only produce new characters or character-combinations by a correlative and a combinative mode of ontogenetic evolution, or by loss of genes, as demonstrated by abundant experiments, but undoubtedly there takes place also a new formation of genes in evolution. This they hold to come about in successive stages through long enduring external influence, which first acts upon the cytoplasm of the cells and especially of the germ-cells. This cytoplasm in itself has been proven to have certain hereditary possibilities (plasmogenous heredity). Under long persistent external influence there form first preliminary stages of genes in the cytoplasm which finally, when a certain "threshold" (Schwelle) of continued strain is passed, become true genes of the heredity-chromatin. When this takes place, mutations appear abruptly (salto-mutations).

This view, here altogether too briefly presented, would explain the absence of evolution through salto-mutations in cases of persistence under continued stable exterior conditions, and since the cytoplasm is known also to influence directly the heredity-chromatin, also the absence of flucto-mutations or variations under stable conditions through lack of external stimulation.

However, in the cases where no new genes are formed by external influences, new characters could still appear through loss of genes or correlative or combinative modes of production of new genes from the old ones within the germ-plasm. This, however, leads to a restrictive cone of divergence (" Streuung ") of the characters and through " self-differentiation " by a combinative mode of gene-production to the excessive characters of many terminal series (*e.g.,* dinosaurians); and to the rigid

persistent terminal types, on the other hand, through the gerontic rigidity of the remaining stock of genes. The principal causes of the persistence of terminal forms would then be the failure of production of new genes arising from the cytoplasm, through external influences, and the senescent rigidity of the remaining genes.

The persistent radicles, on the other hand, correspond to the extreme development of what Salfeld terms "Konservativreihen." There are series in which the salto-mutations appear in very long intervals, while the numerous side-branches (which furnish the index-fossils) develop by rapid salto-mutations. These persistent radicles are therefore able to undergo new periods of explosive and climacteric development ("Virenz-perioden" of Wedekind) and are thus still less absolutely persistent than the persistent terminals. In these conservative series, according to Salfeld, flucto-mutation is so prevalent that sharply defined "species," or better mutants, can not be separated, as notably in the phyla of Phylloceras and Lytoceras which range, qualitatively unchanged in their characters, through Jurassic and Cretaceous time. They thus represent true persistent radicles. This fact, combined with the observation of the vitality, relative primitive simplicity and adaptation to a variety of conditions of persistent radicles, pointed out by the writer in his former paper, suggests that the complex of genes is able to remain relatively undisturbed through external influences (only flucto-mutations appearing) in one part of these groups which persist as radicles, while those parts which become changed through the addition of genes by way of the cytoplasm turn into the side-branches by salto-mutation.

EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

III. The Effect of Successive Etherizations on the Duration of Life of Drosophila [1]

PROFESSOR RAYMOND PEARL AND SYLVIA L. PARKER

Purpose and Plan of Experiments

In any experimental work of a genetic character on *Drosophila,* it is often necessary to anesthetize the flies which are to be used in an experiment for a sufficiently long time so that they may be sexed and sorted into different groups for the purpose of making matings, etc. It has been shown by Morgan (33) that this procedure has no effect upon the causation of morphological mutations, the inheritance of which he has studied (9). The effect might, however, conceivably be quite different in the case of a physiological character like duration of life. Any one who has undergone a major surgical operation feels that anesthetization is at least immediately a rather profound physiological disturbance. Unfortunately, so far as we are aware, no accurate determinations have ever been made to show whether in man one or more anesthetizations changes the expectation of life. As a matter of fact, there are presumably no human data on the point available in any such amount as would be necessary for actuarial determinations, because in man anesthetization is, generally speaking, only undertaken in connection with surgical operations of greater or less severity, so that if we did have statistics of expectation of life of persons who had been anesthetized, there would always be involved the two factors of anesthetization and oper-

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 54. For description of the method of numbering bibliographic citations see the second paper in the series (32).

ation. In *Drosophila* these two factors can be separated.

It has seemed important, in an early stage of our experimental work on the duration of life in this form, to make a careful and extensive experimental test of the question of whether anesthetization singly or repeated changed in any way the expectation of life or form of the life curve, so that if this factor does have any significant influence, either favorable or unfavorable, due allowance may be made for it. It is the purpose of this paper to report the results of such a test.

The flies used in this experiment were flies of line 107 (generation 8 since January 14, 1921, line bred from a single brother and sister mating for approximately 30 generations). The characteristics of this line relative to duration of life have already been described (*cf.* Pearl and Parker (32)). The 4,330 flies used emerged between 10 A.M. April 18, 1921, and 4 P.M. April 22, 1921, from thirty-five mass cultures started in half-pint milk bottles April 7, 1921. The regular procedure in these experiments was to collect the flies from all 35 breeding bottles in one empty bottle and then to count the flies through a counting tube into 1-ounce vials, allowing 50 flies to each vial.[2] Ten vials were used for each series, except the control series, which had 18. For two of the series only two hours were allowed between successive emptyings of the mating bottles, to get flies at an average age of one hour, assuming that they emerged uniformly over the interval. One series was etherized as soon as counted out, and the other series kept as a special control group to see if the handling when the flies were so young and soft had any effect on the duration of life. For the rest of the series the flies were allowed to emerge over a 24-hour interval. Each day's hatch was divided randomly and as equally as possible among the different series.

[2] It will be noted that the totals shown in Table I do not accord exactly with this statement. The discrepancies are due to the fact that a few flies were lost in changing to fresh bottles in the course of the life duration determinations made according to the technique described in (27), and occasionally a bottle was broken by accident and all its contained flies lost.

The counting tube referred to above is a device invented in this laboratory which we find extremely useful in a great deal of the experimental work. It was devised and first used in connection with studies of the growth of experimental populations of *Drosophila* (*cf.* Pearl (7), and Pearl and Kelly (34)). Its construction is shown in Fig. 1.

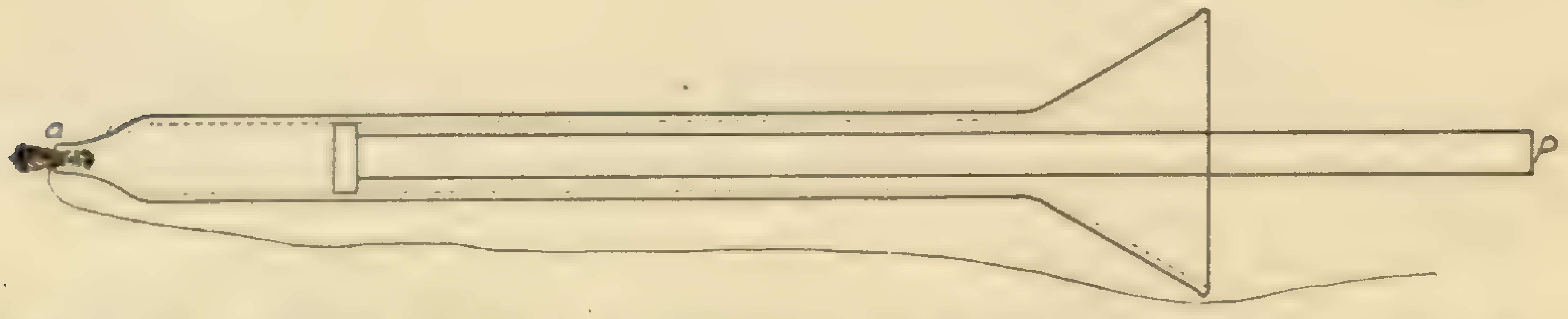

FIG. 1. Diagram showing construction of *Drosophila* counting tube. The aperture at *a* is just large enough to allow one fly to pass through at a time. The essential dimensions are as follows: length over all 25 cm., diameter of main tube 2 cm., diameter of funnel mouth 6 cm.

When it is desired to count a definite number of flies the small aperture *a* is temporarily plugged with a bit of cotton wool, the plunger *P* is removed from the tube and flies are shaken into the counting tube by inverting the open bottle containing them over the funnel mouth of the counting tube. Then the plunger is inserted and gently moved forward to concentrate the flies in the lower end of the counting tube. Then the counting tube with enough cotton around it to close up the mouth of the bottle is inserted into the bottle into which it is desired to place the counted flies and the plug removed from the aperture *a*. Then as the flies come out of the tube, one by one, through the aperture *a*, they are counted as they pass this point, with the aid of a tally register, such as is used by doorkeepers at theaters, etc. The plunger is gently moved forward as necessary to keep up an even flow of flies through the mouth of the tube.

The ether dose used was constant for all the flies throughout the experiment. The group to be etherized was shaken into a clean half pint milk bottle; 5 c.c. of ether was poured onto a piece of absorbent cotton fastened to the under side of a cork stopper; the bottle with

the flies was stoppered tightly with the cork and left for two minutes. Then the flies were turned out on a tile and sexed and counted (since that operation corresponds in extent of handling to what we need to do in making up matings, etc.), then emptied into a vial with fresh food, where they recovered from the ether in about half an hour. For each successive group of flies a fresh bottle and fresh cotton for the ether were of course used.

In all other, here unspecified, particulars the technique used in these ether experiments was uniformly that described in detail in the first paper of this series (27).

Seven series of experiments were conducted, differing in respect of the number of times the flies were etherized, and in their age at the time of etherization. The seven series were as follows:

A. Etherized once when one hour of age.

B. Etherized once when twelve hours of age.

C. Etherized once when thirty-six hours of age.

D. Etherized once when three and a half days of age.

E. Etherized twice when seven, and fourteen days of age, respectively.

F. Etherized three times when seven, fourteen, and twenty-one days of age, respectively.

G. Etherized four times when seven, fourteen, twenty-one and twenty-eight days of age, respectively.

DATA

The l_x lines for the several series of etherized flies and the controls are given in Table I. These l_x distributions are calculated on the basis of 1,000 flies at emergence from the pupal stage, with the absolute number of flies on which the distribution is based given at the bottom of the column in each case.

The l_x distributions for all etherized flies and for their controls in the ether experiment, and for two tests of the flies in line 101 and its continuation 107, are shown graphically in Fig. 2. The data for the survivorship lines in the two tests of line 107 are to be found in Pearl and Parker (27).

TABLE I

SURVIVAL DISTRIBUTION OF ETHERIZED AND NON-ETHERIZED DROSOPHILA CULTURES

Age in Days	Etherized Series								All Controls	Controls 1 hr. Old	Controls 12hrs Old
	A	*B*	*C*	*D*	*E*	*F*	*G*	All Etherized			
1– 6	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000	1,000
7–12	988	998	988	998	993	990	995	993	985	990	983
13–18	986	993	978	995	984	983	995	988	980	987	976
19–24	984	971	978	984	971	971	964	975	974	981	970
25–30	970	953	966	965	957	918	910	949	925	918	928
31–36	942	916	912	942	944	910	857	918	903	895	907
37–42	902	880	856	926	901	879	810	880	854	843	859
43–48	778	722	796	847	793	763	698	771	735	753	724
49–54	629	634	732	725	643	598	605	652	604	701	550
55–60	453	492	625	581	465	462	476	507	454	554	398
61–66	117	95	397	301	153	249	145	183	150	138	157
67–72	47	34	136	116	79	165	71	92	53	71	43
73–78	12	9	29	37	22	65	17	27	15	19	13
79–84	0	0	0	0	0	0	0	0	0	0	0
Absolute number of flies....	428	443	411	432	445	413	420	2,992	1,338	478	860

It is at once evident, from an examination of the figures in Table I, and the diagram, that there was no considerable difference in duration of life, or in the form of the life curve, for the etherized flies taken as a class, and the non-etherized groups. It is, however, desirable to examine the results of the experiments in detail in order to see whether there are detectable by biometric methods any small but still statistically significant differences between the several groups. To this end, Table II has been prepared, giving the usual biometric constants for the several series.

Comparing first the entire etherized group as a whole with those which never had any ether at all in their lives, it is seen that the mean duration of life (expectation of life at emergence from pupa) is $1.82 \pm .30$ days longer in the former (etherized) than in the latter (normal) group. The difference is thus slightly more than 6 times

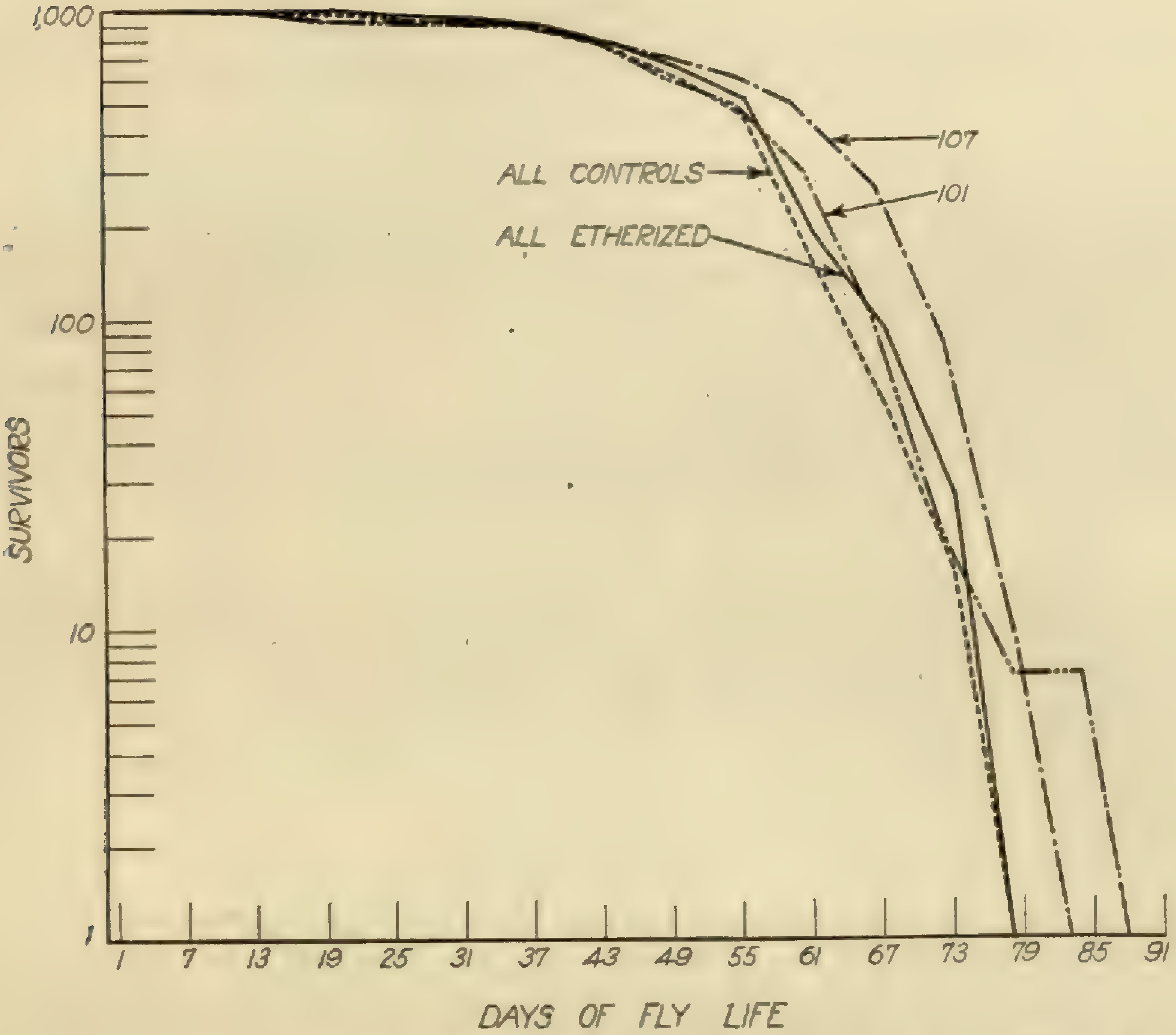

FIG. 2. The l_x lines for all etherized and controlled flies, plotted from the data of Table I.

TABLE II

BIOMETRIC CONSTANTS FOR DURATION OF LIFE OF ETHERIZED AND NORMAL DROSOPHILA

Treatment	Number of Flies	Mean (in days)	Standard Deviation (in days)	Coefficient of Variation
All etherized	2,992	51.60 ± .16	13.30 ± .12	25.77 ± .24
All controls	1,338	49.78 ± .25	13.68 ± .18	27.47 ± .38
Etherized when 1 hour old	428	50.82 ± .38	11.76 ± .27	23.14 ± .56
Etherized when 12 hours old	443	50.20 ± .39	12.25 ± .28	24.41 ± .59
Etherized when 36 hours old	411	53.36 ± .46	13.91 ± .33	26.06 ± .65
Etherized when 3½ days old	432	54.50 ± .40	12.36 ± .28	22.68 ± .55
Etherized when 7 and 14 days old	445	51.43 ± .40	12.50 ± .28	24.31 ± .58
Etherized when 7, 14, and 21 days old	413	51.72 ± .50	15.01 ± .35	29.03 ± .74
Etherized when 7, 14, 21, and 28 days old	420	49.26 ± .47	14.42 ± .34	29.26 ± .74
Controls (taken out of mating bottles when 1 hour old)	478	51.11 ± .42	13.75 ± .30	26.90 ± .63
Controls (taken out of mating bottles when 12 hours old)	860	49.04 ± .31	13.58 ± .22	27.69 ± .48

its probable error, and must therefore be regarded as statistically significant. Absolutely, however, the difference is small. It is equivalent to only 3.7 per cent. increase of the expectation of life of the controls. In variability in respect of duration of life there is plainly no significant difference between etherized and control groups.

It is not entirely clear that the small difference between the etherized and control groups in mean duration of life can be regarded as due to the influence of the ether. An examination of the last two lines of Table II shows that an entirely similar difference in the means appears between the two control groups, which differ only in respect of the time when they were taken from the mating bottles, and without either having been etherized. The difference in these two means amounts to $2.07 \pm .52$ days, a statistically significant and absolutely slightly larger difference than that between etherized and control groups. Again there is no significant difference in variability in the two groups.

Altogether we shall be justified in concluding that there is no evidence from these experiments that the occasional etherization of *Drosophila* to the extent necessary in sexing and making matings alters the expectation of life by an amount large enough to introduce any sensible source of error into experiments on the duration of life in this form, except possibly where the most careful and accurate actuarial determinations need to be made. Then it will be well to have this possible source of error in mind and to plan the experiments in such way as to check it.

Examining the results for the different etherized series it is seen that the highest mean duration of life appears in the group etherized once at 3½ days of age, and next to this stands the group etherized once at 1½ days of age. Both of these give relatively high mean values. There also appears a definite, though not particularly marked tendency for the variability in duration of life to be greater in the groups which were etherized several

times. No great importance is probably to be attached to these differences between the several groups, however, though some of them appear significant statistically.

Conclusion

From the experiments herein described, involving the determination of the total duration of life in 4,330 individual flies, it may be safely concluded that no sensible error will be introduced into duration of life experiments on *Drosophila* as a result of completely anesthetizing the flies with ether, at least up to as many as four times in the course of their lives.

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SHORTER ARTICLES AND DISCUSSION

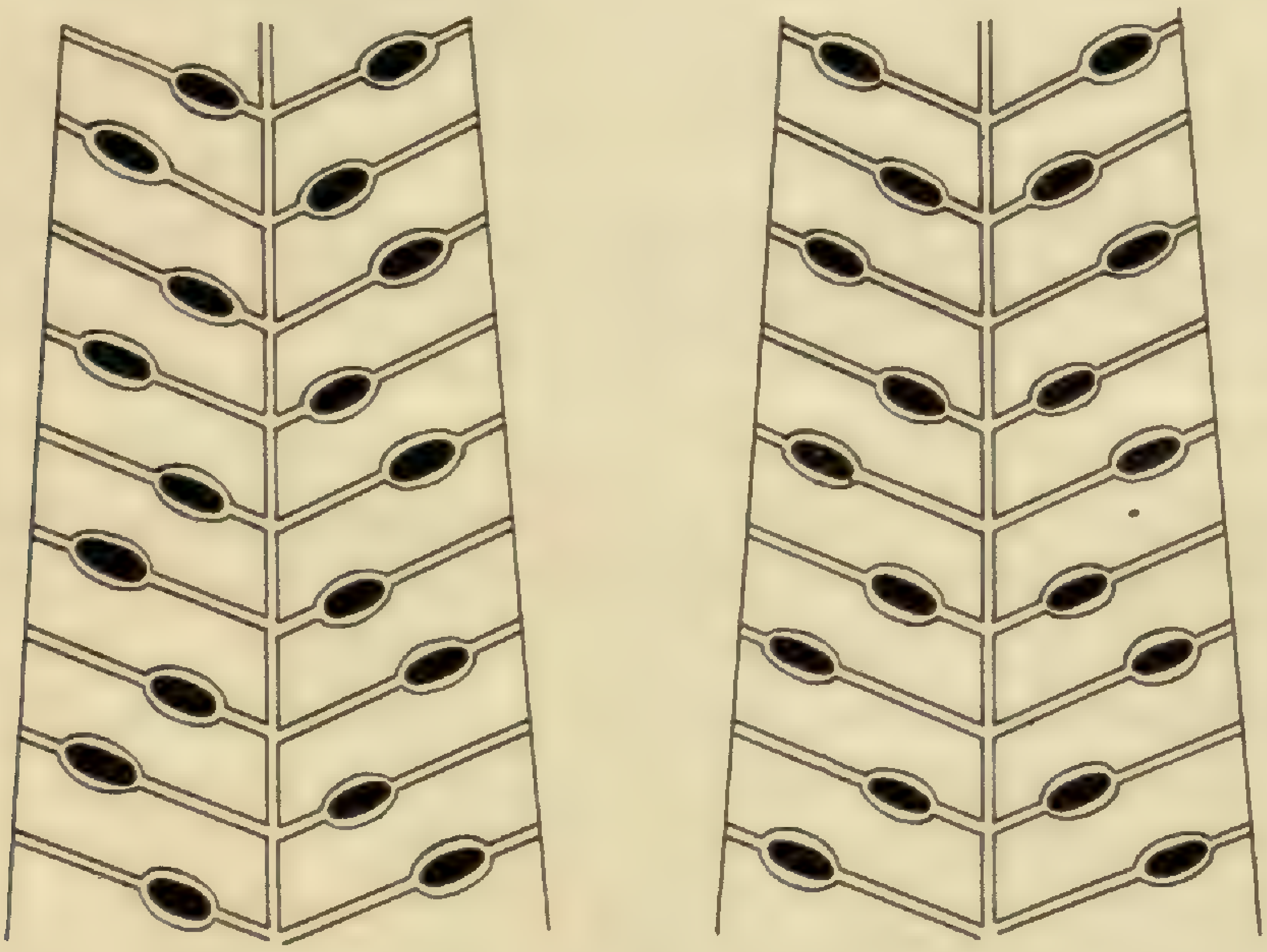
A TEACHING NOTE ON THE ARRANGEMENT OF THE TUBE-FEET IN ASTERIAS

SEVERAL summers ago while directing the laboratory work on Echinodermata in the Invertebrate Course at Woods Hole, a question was raised by one of the students as to the correctness of the account which had been given of the arrangement of the tube-feet in the common starfish, *Asterias forbesi* Desor. An examination of the point in question revealed the occurrence of a rather interesting irregularity which is here briefly reported on. The source of the conditions here described is entirely unknown, but inasmuch as this starfish is commonly used as material for laboratory study, it occurred to me that the facts themselves might be of interest to teachers of invertebrate zoology.

It will be recalled that in *Asterias* the tube-feet are arranged in four longitudinal rows, two of which are on each side of the radial canal (the mid-ventral line of the arm). The tube-feet in these rows of two are arranged in an alternate manner, nearer or farther from the mid-line, thus allowing for the accommodation of more tube-feet in a given linear space. The tube-feet are connected with the radial canal by short transverse canals, which are thus longer or shorter according as they pass to tube-feet in the inner or outer series. This arrangement of the tube-feet can be clearly made out in properly dried specimens from which the remnants of the tube-feet themselves are all removed. Their position is clearly marked in such a preparation by the perforations between each pair of ambulacral ossicles, and the whole topography of the ambulacral groove is well demonstrated. It is usually stated that[1] "Each pair of transverse canals consists of a short canal on one side and a longer canal on the opposite side of the radial canal. The short and long canals of each side are alternating." This arrangement of the tube-feet is shown in a diagrammatic way in Fig. 1, in which the tube-feet are represented as black ovals situated in the perforations be-

[1] Quoted from Petrunkevitch, "Morphology of Invertebrate Types," New York, 1916, pages 177 and 178.

tween adjacent ambulacral ossicles. This, the common arrangement, may be designated Type I. It will be noted that as one runs along the arm the transverse canals of succeeding pairs are long-short, short-long, and so on.



FIGS. 1 AND 2

However, it appears from my experience in this laboratory that some teachers give a different description of the arrangement of the tube-feet. According to these teachers the length of the transverse canals does not alternate in a single pair, but is the same on both sides of the radial canal. This would lead to an arrangement which is shown diagrammatically in Fig. 2. According to this account as one runs along the arm the transverse canals of succeeding pairs are long-long, short-short, and so on. It seemed worth while from a teaching standpoint to determine which of these descriptions is the correct one. For convenience, the arms of the starfish will be named in the conventional manner *a, b, c, d, e*—*a* being the first arm to the right of the madreporic plate (as seen from the aboral surface), the others being named in a clock-wise direction around the disc.

In all, seventeen specimens of *Asterias forbesi* have been examined, some more completely than others. The first two or three pairs of tube-feet at the very base of the arm are usually rather crowded by the abrupt narrowing of the ambulacral groove, so that it is rather difficult to say exactly to which type

of arrangement they belong. They seem usually to be more like Type II than Type I. The groove widens rapidly, however, and the four characteristic rows are quickly established. In the majority of cases the arrangement is undoubtedly like Type I, and this is obviously the source of the usual text-book description.

However, in at least nine specimens of the seventeen examined, one or more arms have the Type II arrangement. This may occur in any arm, but in my specimens is most frequent in arm *e* (five cases). Sometimes the Type II arrangement is established from the very beginning of any regularity at the base of the arm; in my specimens there were three (probably four) cases of this kind in arm *e* and one in arm *d*. In such cases the Type II arrangement may persist throughout the entire length of the arm. More commonly, however, the Type I arrangement is first established and after persisting for a longer or shorter distance abruptly changes to Type II. The number of Type I pairs in such cases seems usually to be small. The transformation is made by a slight irregularity on one side such that two long or two short transverse canals are adjacent—and thereafter the arrangement is again entirely regular. Sometimes the region of change is more irregular, but never strikingly so. In one case, in arm *a*, the arrangement was first like Type I, soon changed to Type II, and in the distal part of the arm changed back again to Type I. In the seventeen specimens examined the Type II arrangement has been found to occur (in some part of the arm) as follows:

Arm *a*	3 cases
" *b*	3 cases
" *c*	2 cases
" *d*	1 case
" *e*	5 cases

The number of arms with Type II arrangement in any one individual varies considerably, the results for my specimens being as follows:

1 arm affected	7 cases
2 arms affected	1 case
5 arms affected	1 case

In two cases, both arm *a*, Type II was found to occur in regenerating arms, though near the base the Type I arrangement

occurred. Whether the injury to the arm was the source of the change is not apparent.

It will be seen, therefore, from the above account that both descriptions of the tube-feet arrangement are correct, but that the one usually given in text-books (Type I) is by far the more common; furthermore, that the one type may change to the other with no apparent structural reasons for the transformation.

The facts here presented furnish, I believe, a complete explanation of the difference in the laboratory accounts as given by different teachers.

ROBERT H. BOWEN

MARINE BIOLOGICAL LABORATORY,
WOODS HOLE, MASS.

THE MICRO-FILTER FOR MINUTE FLAGELLATES

IT is frequently desirable during the study of the minuter protozoa, and especially of the small flagellates, to concentrate the organisms. This the writer has been able to do in a very simple and satisfactory manner by means of the device shown in Fig. 1, which may be called the micro-filter; a name applied not only because of its office, but also because of the minute piece of filter-paper used.

The contrivance consists of a standard, either of wood or of metal, which supports a burette tube, a minute circle of filter-paper, and a vessel beneath. The water containing the protozoa to be concentrated is introduced into the burette from above, by means of a funnel, and the pinch cock (*O*) opened sufficiently to allow the liquid to drop into the small funnel or circle of filter-paper beneath (*P*). The filter is supported by means of stout copper wire. The flow of water from the burette can be nicely regulated by means of the pinch cock, which, to give the best results, should be of the screw variety. The water drops through a glass tube, drawn out into a fine point (*T*). It was found convenient to have several of these tips of different diameters.

Considerable experimentation is necessary before the exact balance between the flow of water from the burette and that from the base of the filter-paper funnel can be secured. When this balance is reached, the burette is filled and the water allowed

to filter into the vessel on the base of the stand. It is necessary, at approximately fifteen-minute intervals, to thrust into the burette, as far down as the shoulder, or point of taper (just above the rubber tube on which the pinch cock rides), a straight

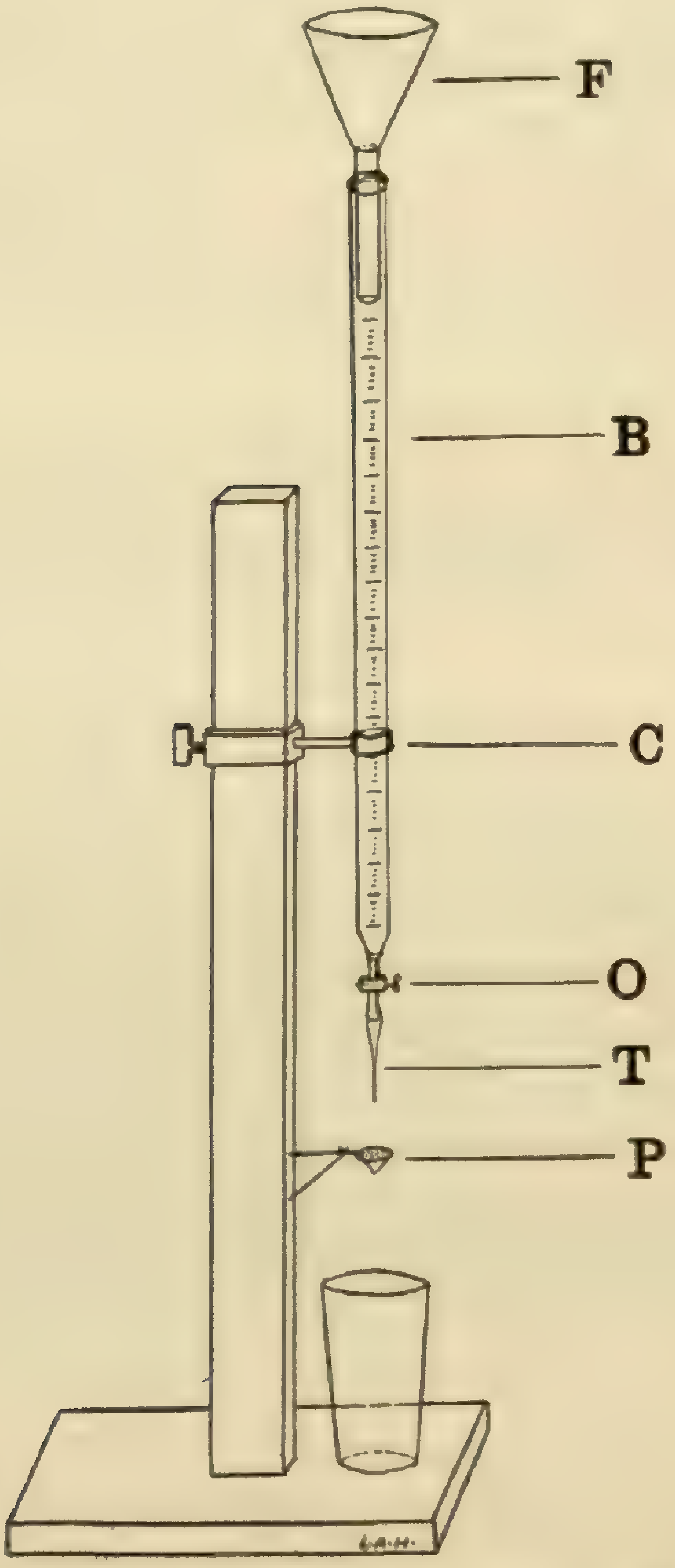


FIG. 1. The Micro-filter. Simple wooden stand for the micro-filter, supporting: funnel (*F*), burette (*B*), clamp for holding burette (*C*), pinch cock (*O*), capillary tip (*T*), filter paper (*P*), and vessel for catching filtered water beneath.

FIG. 2. Pipette with flattened tip for scraping filter paper, to remove filtered organisms.

copper wire rod, holding in its lower end a bit of cotton. This serves to stir up the material which it is desired shall be deposited upon the filter paper, to prevent it from settling and adhering to the sides of the glass, on the slopes of the taper.

When the entire amount of water has passed through the filter-paper, the latter is removed, spread out, and immersed in a bath of water, in a watch crystal. The water should just cover the filter-paper.

The device shown in Fig. 2 is now brought into play. This consists of a glass pipette, flattened and spread at its tip, and serves admirably for gently scraping and sucking the surface of the filter-paper, as it lies in the watch crystal. This withdraws into the pipette the organisms which have been filtered out. These can now be transferred to a glass slip and examined under the microscope, or injected into culture media as inoculations.

The writer has found that, with practice, the possibilities of the micro-filter may be extended to aid, in many ways, in the study of the protozoa.

LEON A. HAUSMAN

CORNELL UNIVERSITY

COMPLETE LINKAGE IN *DROSOPHILA MELANOGASTER*[1]

IN 1917 a mating appeared in the cultures of the authors, the flies from which showed no crossing over in the region scute to forked of the sex chromosome, although the factors echinus, cut, vermilion and garnet, were between the extreme points. This culture appeared spontaneously; selection played no part in it. The stock from this culture has now passed through not less than 80 generations and numbers over 3,000 matings. During this time no crossing over has appeared within the known length of the sex chromosome.

In experiments including the second chromosome points, black and purple, it has been shown that no crossing over takes place between these points when complete linkage exists for the first chromosome. Likewise the third chromosome points, dicheate and hairless, have shown complete linkage when the points scute to forked in the first chromosome, and the points black to purple in the second chromosome show the same phenomena.

The disturbing cause is genetic, behaving as a recessive. Its

[1] Papers from the Biological Laboratory, Maine Agricultural Experiment Station, No. 142.

position is in the region of dicheate hairless of the third chromosome. It may be noted that such recessive factors effecting the mechanism of segregation show what might be called delayed Mendelian results for the F_2 flies must be tested for their linkage relations before anything can be said regarding the stock.

Complete linkage has been reported in but one other case. Thus in 1912 Morgan showed that crossing over did not occur in the second chromosome of the male of this same species, *melanogaster*. This phenomenon has since been extended to include the other chromosomes. If it be considered that crossing over as originally discovered for the female of this species is the normal, then Sturtevant has shown not less than three dominant factors to materially reduce the normal amount of a crossing over in the second and third chromosomes. A further incompletely analyzed case of the same investigator suggests that a third chromosome dominant partly controls an increase in crossing over in the second chromosome. Crossing over variations have been shown by Bridges in his "deficiency" case, etc. From this it appears that there are three kinds of effects shown by the crossover mechanism. The first case, that of Morgan, shows no crossing over in the male. No genetic factors have as yet been shown to be responsible for this. The second case, that of Sturtevant, shows genetic dominant factors responsible for reducing crossing over in the female. The third case, given here, shows recessive genetic causes allowing no crossing over in the female. It further shows these factors capable of acting on chromosomes of which they are not a part.

Detlefesen and Roberts using the sex-linked factors, white and miniature, present another kind of evidence. In a selection experiment they show crossing over to decline from the normal amount (about 33 per cent.) to nearly zero per cent., no evidence being presented as to the causative agent, although the suggestion is made that "crossing over in the various regions of the sex chromosome (and the other chromosomes?) is probably controlled by multiple incompletely dominant factors." From what has been indicated above it seemed more probable that recessive factors, perhaps one, are responsible for these linkage variations. Especially is this true of their results in series A and A^1, for with delayed Mendelian segregation, recessive autosomal factors effecting crossing over in the

sex chromosome, mass mating in every other generation, and complications resulting from only being able to test the female, it is to be expected that selection will progress slowly at first and come suddenly to the climax of reduced crossing over.

Marie S. Gowen,
John W. Gowen

Orono, Maine

THE

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EXPERIMENTS WITH ALCOHOL AND WHITE RATS

EDWIN CARLETON MACDOWELL

STATION FOR EXPERIMENTAL EVOLUTION, COLD SPRING HARBOR, LONG ISLAND, N. Y.

SINCE the familiar paper by Elderton and Pearson ('10) upon the physique and ability of children from alcoholic parents, much discussion has taken place on the relation of parental alcoholism to the condition of the offspring. A small proportion of this has been based upon experimental work with animals, as that of Stockard ('12 and '13), Stockard and Papanicolaou ('16 and '18), Nice ('12 and '13), Pearl ('17), and Arlitt ('19). From such studies there should be no hope of obtaining an immediate analysis of the human problem. In so far as alcoholism in man is sociological, involving factors of family life, environment and education, no study of laboratory animals can have significance. The way such studies may have a bearing upon the human problem is through the revelation of general biological reactions that may in all the animals available for study, be found so invariable that it becomes safe to conclude that they appear in man as well. How far the specific findings herein reported for white rats may apply to different animals is a matter for experiment and not conjecture. But even were such a biological analysis secured, the other phases of the human problem would not be solved.

From the data at hand are there any indications of general biological reactions that may have significance for all animals? Stockard and Papanicolaou, with guinea pigs, found that alcoholization of parents gave un-

favorable results in the offspring; Pearl reported generally favorable results in the offspring of treated fowl; Arlitt reported unfavorable results from mild doses on rats, while Nice, also with mild doses, found his test mice slightly better in growth and fertility but less active, as measured by the revolutions of the revolving cages in which they were placed, than the controls. Earlier, Hodges ('03) had found the viability of puppies reduced by the treatment of their parents; the treated dogs were less active and more susceptible to distemper; Laitinen ('08) reported high rates of death at or soon after birth of guinea pigs and rabbits from treated parents.

Accepting these general statements as correct, there appears to be no obvious uniformity in the results obtained by different investigators. But this lack of uniformity may be only apparent; it is possible that not all the results as presented will be confirmed by subsequent investigations since none of the experiments reported have escaped unfavorable criticism from some standpoint. Alcoholism has such a multiplicity of aspects that it is a matter of great difficulty to arrange experiments concerning its effect on the offspring of treated animals that will be beyond criticism. For technique satisfactory to a physiologist may involve serious errors in the eyes of a psychologist, while the experiments of both may, to a geneticist, seem to have weak points. Until alcohol studies meet the requirements of all critics no final conclusions can be reached. In problems involving comparisons between experimental and control individuals the nature of the controls is no less important than the comparison itself. However true this appears to be for all experimental work, it is surprising to note that the main adverse criticisms of the experimental studies of the influence of alcohol upon the offspring have been aimed at the controls.

In spite of the general lack of uniformity in the results as they stand, at least one criterion appears to show consistency. This is the reproductive capacity of the treated

individuals. All the experiments appear to indicate an immediate reduction in the number of offspring. The uniformity of this result tends certainly to increase its value as a *general* result; but even so, as long as the controls are subject to criticism, the apparent consistency may be due to the controls and not to the regularity of the reactions to alcohol. For a single result can not at the same time prove the reliability of the controls and the results of alcohol treatment. It is hoped that the controls employed in the following experiments will be found to approach the ideal of satisfying all requirements.

Methods

In 1914 an investigation was undertaken upon the influence of alcohol on the untreated descendants of white rats with the primary object of studying the behavior, or learning capacity, in different generations. In the summer of 1917 war conditions necessitated repeated reductions of the stocks until, by the end of the next year, the material was completely lost. This calamitous termination of the work must be borne in mind, for, in spite of the final nature of this report, the data come from an investigation that was not completed.

Material and Breeding.—The rats employed belonged to four strains; three of these strains originated respectively from three pairs of rats in the Wistar Standard Stock, the fourth strain had been bred in this laboratory for three generations. All matings were between full brothers and sisters. When 28 days old the litters used to start these experiments were divided into two lots on the basis of equal weight and equal numbers of each sex; one of these lots was used as controls, the other was treated. All matings were between the original treated males and females or their descendants, or between the original control males and females or their descendants. In each generation the control matings parallel those of the descendants of the treated animals, so that each group of test animals in each generation had its own particular

group of controls. Since inbreeding was the rule, the closest possible relationship for the tests and controls in the successive generations was secured; they came from a single pair of grandparents or great-grandparents, and were thus raised at the same time, and after the same number of generations of inbreeding.

Treatment.—The treatment of these rats was by means of the inhalation method, now made familiar by the work of Stockard and Pearl. The rats were placed in closed tanks filled with alcohol vapor; these tanks have been described in detail elsewhere (MacDowell and Vicari, '21). Beginning at weaning (28 days) the rats to be treated were placed in the tanks for 30 minutes a day for 7 days. After this the duration of the daily treatment was measured by the reactions of the animals; for the next 14 days the rats were left daily in the fumes until they were obviously under their influence; subsequently the rats were left each day until they were completely anesthetized. This required from three to four hours for the older rats.

Criteria.—The term *treated* is used to indicate rats that were placed in the alcohol fumes after birth. The following generations are herein reported: (1) the treated rats, (2) the treated offspring, (3) the untreated offspring, (4) the untreated offspring of (3) (second untreated generation following one treated generation). For these rats the following types of data are given: the behavior in the maze, as measured by time per trial; behavior in a multiple choice apparatus, measured by the number of correct first choices; fertility, judged by the size of the litters and the number of litters; body weight, as judged by growth curves based on weekly weighings.

MAZE-BEHAVIOR

Apparatus and Training.—The maze used in this study was built according to the details given by Watson ('14); namely, a concentric arrangement of five alleys with doorways and blind alleys so arranged that the true path from

the outside to the center required a rat to turn alternately to the left and the right at successive doorways. A rat's training was started at the age of 56 days, after preliminary feeding in the center of the maze on each of the 7 preceding days. Three successive trials a day were given. After the first and second trials the rat was removed from the center as soon as it had tasted the food (bread and milk) which was always found there; after the third trial, it was allowed to eat for five minutes. This training was given for eight successive days. The observations were so automatic that there was practically no possibility that the results were being influenced by an unconscious bias on the part of the observer. In the case of the treated rats the alcohol was given each day following the trials in the maze.

Results.—The average time per trial for each day of the training of the different groups of rats is represented in Fig. 1. The test rats, whether actually treated, or the descendants of treated rats, are represented by the broken lines, and their respective controls by the solid lines. The numbers of rats included in the different curves, beginning at the left, are as follows: 55 treated rats and 62 controls; 46 tests and 48 controls; 25 tests and 25 controls; 8 tests and 20 controls. The broken lines tend to lie above the solid lines. The tests tend to give higher time averages than the controls, that is, the tests took longer time to run a trial. The inferiority shown by the treated offspring from treated parents (fourth pair of curves), and by the untreated offspring from untreated parents and treated grandparents (third pair of curves) is of the same order of magnitude as that shown by the treated animals themselves; untreated offspring from treated parents show less inferiority than their own untreated offspring. Considering the significance of the differences between the tests and controls for each day independently, the following results are found: the differences between the tests and controls are over three times their probable errors on five days in the

first pair of curves, on no day in the second pair, on four days in the third pair and one day in the fourth pair. All the significant differences favor the controls. However, more important than the significance of individual

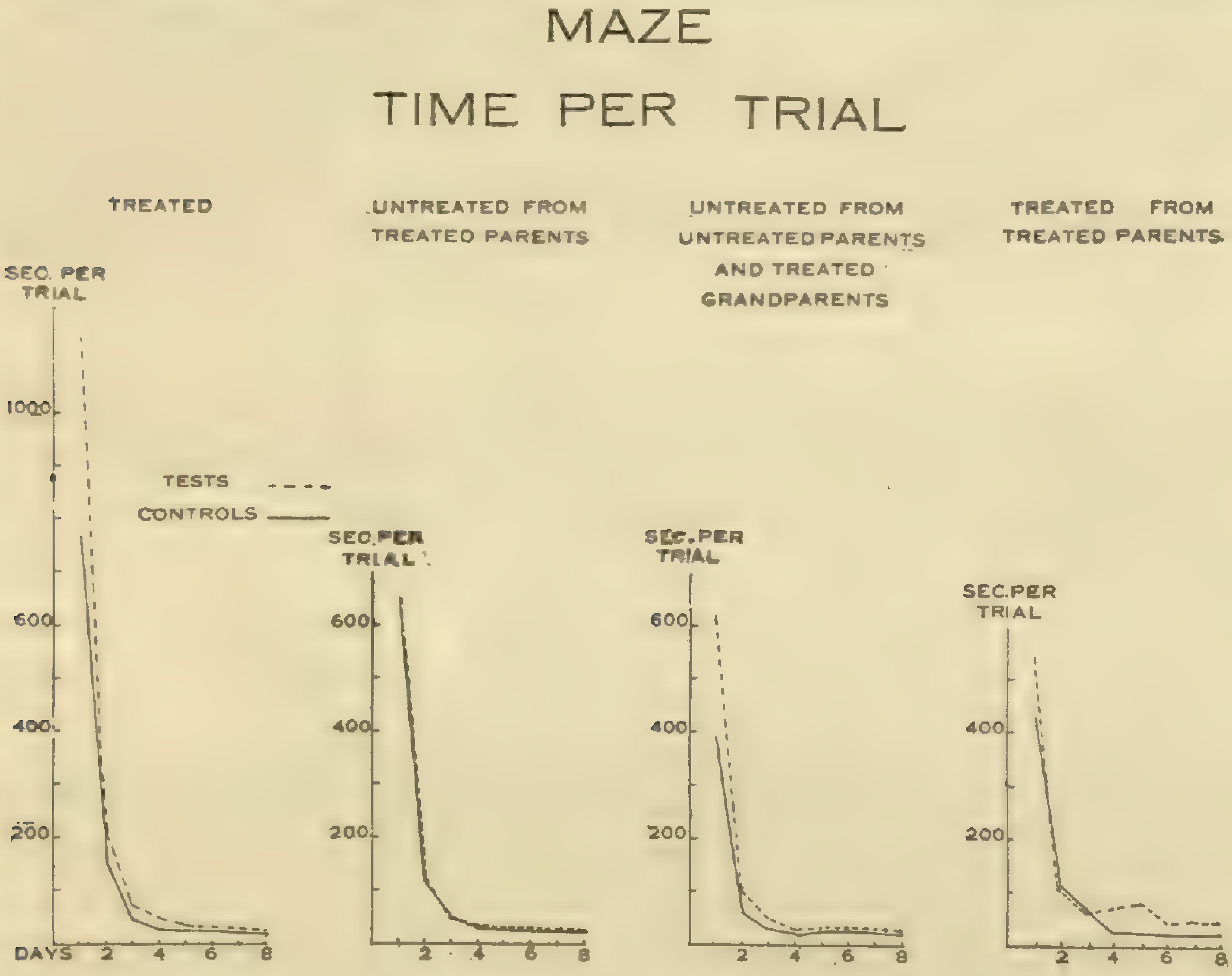

FIG. 1. Comparisons of time averages in four groups of rats—those treated, their treated and untreated children, and their untreated grandchildren. (Data for the third set of curves taken from MacDowell and Vicari, '21, p. 233.) Broken lines tests, solid lines controls.

differences as measured by the probable errors, is the agreement in the direction of the differences on successive days. The fact that the differences on eight successive days lie in the same direction probably has more significance than that half of these taken separately may be significant as judged by their probable errors. Considering the signs alone, in all the curves there are three out of the 32 points of comparison showing the test averages lower than the controls. One of these cases is on the third day of training of the untreated rats from treated parents, the other two cases are on the second and third days of training of the treated rats from treated parents. If chance alone is working, the probability of

eight days giving differences in the same direction is the same as the probability of eight coins coming down all heads; in the long run this will happen once in 256 tosses. The chances of seven out of eight, 1 to 32, of six heads out of eight, 1 to 9. Carrying this comparison further by considering all the generations together, the chances of finding three cases favoring the tests out of thirty-two are in the neighborhood of 1 to 860,000. From all this it appears that the test rats are different, as a group, from the controls. Apparently the only difference between the tests and controls that could explain this result is the alcohol treatment given directly, or in the ancestry of the test rats; this leads to the conclusion that the difference in behavior is due to the alcohol treatment.

Behavior in the Multiple Choice Apparatus

The difference in the behavior of the tests and controls in the generation of the untreated offspring of treated parents is further shown by the training on the multiple choice apparatus. This is the only generation from which sufficient data were gathered for the analysis of behavior on this apparatus.

Apparatus and Training.—The apparatus used in this training consisted of a linear series of nine compartments, with front and back doors operated at a distance by the observer (see Yerkes, '21, for history and uses of this apparatus). Different sets of front doors were opened for the successive trials and the rat was given its reward of food by raising the back door when it entered the "correct" compartment. The "correct" compartment was the one at the extreme right or left (according to the problem) of the series with open front doors. In successive trials, therefore, the correct compartment was never the same one, and the solution of the problem did not depend upon the repetition of a regular kinesthetic habit. The steps in the training were these: at the age of 65 days the preliminary training

was started; on the first two days the doors were all left open and food was exposed to view in every compartment; the rats in groups of five or so were left to run at random in the apparatus. On the second two days the front doors were all open as before, but the food was concealed by covers fastened to the back doors, and when a rat entered any compartment the food was revealed by opening the back door; the rats were run singly on these two days and given ten such feedings a day. On the last two days of the preliminary training only the regular series of doors were opened, but the rats were fed on entering any compartment (20 trials).

Right-hand Problem.—In the first problem the rat was fed only when it entered the right-hand compartment of any set-up (those open in any trial); after wrong choices the rat was confined in the compartment for half a minute, and then, by raising the front door, was permitted to make further choices (10 days, 100 trials); next, the same problem was given with a different series of open doors (2 days, 20 trials). Further training was given in the form of a problem in which the correct door was the open one at the left end of the open series, but the results from this problem are so complicated that they will not be treated at this time. The main reason for this complication is the fact that at the end of the time allotted for the mastery of the first problem the test and control rats exhibited different degrees of perfection; some had made considerable progress in learning, while others had made very little advance. Accordingly, when the reverse problem was given, those that had learned the most were handicapped by the habit already acquired, while those that had not formed the required habit in the first problem were able to progress more rapidly in learning the second problem.

Results.—From a study of the individual reaction tendencies as revealed in the last two days of the preliminary training before the problem was presented, and in the regular training after the presentation of the prob-

lem necessitated the use of the trial and error method of finding the correct compartment, it appeared that the test rats continued the same tendencies in the regular training that were initiated in the preliminary training, but the controls, on the other hand, modified their reaction tendencies as soon as the regular training was started. This result is brought out by the curves in Fig.

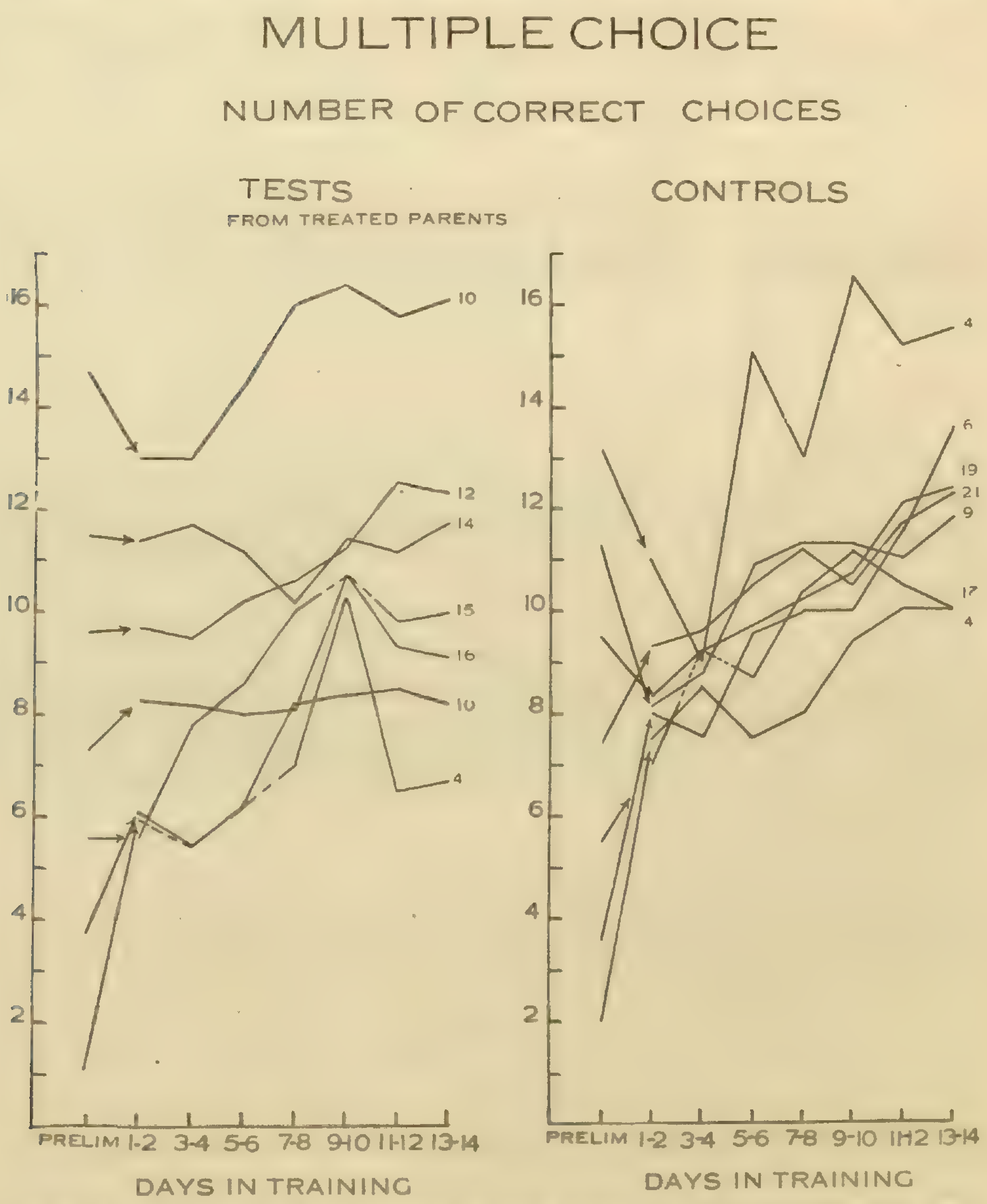

FIG. 2. Showing the relationship for rats from treated parents between preliminary and subsequent performance in the multiple choice apparatus. Average numbers of correct first choices are shown for each successive set of 20 trials. The rats have been classified into groups according to their preliminary records. The figure shows that the behavior of the tests in the preliminary trials is a fairly good index of their behavior in the regular training, but the behavior of the controls in the preliminary trials gives very little indication of the later behavior.

2. The test rats have been classified into seven groups according to the number of right-end choices in the last twenty trials of their preliminary training. The first points of the lines given for the tests indicate the average number of right-end choices made by the rats in each of the groups in the preliminary training; the following points give the average numbers of correct (right-end) choices made by these same rats in successive sets of 20 trials in the regular training. Since the procedure in the regular training is essentially different from that in the preliminary trials, the lines connecting the first and second points are drawn as arrows. The numbers at the ends of the lines give the numbers of individuals included in each group. The arrangement of the controls follows the same plan. Whereas the curves for the tests show a general parallelism, those for the controls are, with the exception of the group of four rats whose preliminary training gave between 12 and 14 right-end choices, relatively independent of the preliminary records. This matter can be brought out more clearly by a study of the coefficients of correlation between the preliminary record of each rat and the trials in the regular training. When the correlation coefficients between the preliminary records and the first 20 trials in regular training, and between the preliminary and the second twenty trials in regular training, etc., are calculated, the figures in Table I are obtained. In every case the differences between the coefficients of the tests and controls (fourth column in Table I) show that the tests have higher correlations, and in all but the correlation between the preliminary trials and the last set of twenty trials in regular training, the differences are statistically significant. These results indicate that there is a real difference between the tests and controls in the way they react to the necessity of using trial and error methods; this may be due to a difference in responsiveness to changes in the situation. The tests appear to be less responsive to the changed procedure, since they continue the same general behavior

as in the preliminary training, whereas the controls modify their behavior as soon as the change is made in the procedure.

TABLE I

CORRELATION COEFFICIENTS, SHOWING THE DEGREES OF SIMILARITY BETWEEN THE NUMBER OF RIGHT-END CHOICES IN THE LAST 20 TRIALS OF THE PRELIMINARY TRAINING AND THE CORRECT CHOICES IN EACH SUCCESSIVE SET OF 20 TRIALS IN THE SUBSEQUENT TRAINING IN THE MULTIPLE CHOICE APPARATUS.

Trials Correlated	Correlation Coefficients		Difference	*D*/P.E.
	Tests	Controls		
Preliminary by 1st 20 trials....	.688±.039	.139±.063	+.549±.074	7.4
by 2d " "	.628±.045	.072±.075	+.556±.087	6.3
by 3d " "	.592±.048	.339±.067	+.253±.082	3.1
by 4th " "	.444±.060	.070±.075	+.374±.096	3.8
by 5th " "	.432±.061	.101±.074	+.331±.095	3.4
by 6th " "	.489±.057	.049±.075	+.440±.094	4.6
by 7th " "	.342±.061	.212±.072	+.220±.094	2.3

All the coefficients are positive; the plus sign is used before the differences to indicate that the coefficients for the tests are higher than the corresponding ones for the controls.

In view of the above, the direct comparison of the averages of the tests and controls in regular training would lead to error unless the average performance in the preliminary training happened to be the same for both sets. In the long run this would undoubtedly be the case, but, as it happens, the averages for the tests and controls do not agree in the preliminary training. However, it was found that this difference depended upon the rats with strong right- or left-hand tendencies, for if these (those tests and controls with more than 12 or less than 3 right-end choices in the preliminary training) be omitted, the average of all the rest of the rats was the same for the tests and controls. Using the rats whose preliminary records showed between 3 and 12 inclusive right-end choices, the averages for the curves in Fig. 3 were obtained. Starting with the same average tendency to enter the right-end compartment in the preliminary training, the controls

increase the number of correct first choices more rapidly than do the tests, and as the difference between the averages increases it becomes statistically significant.

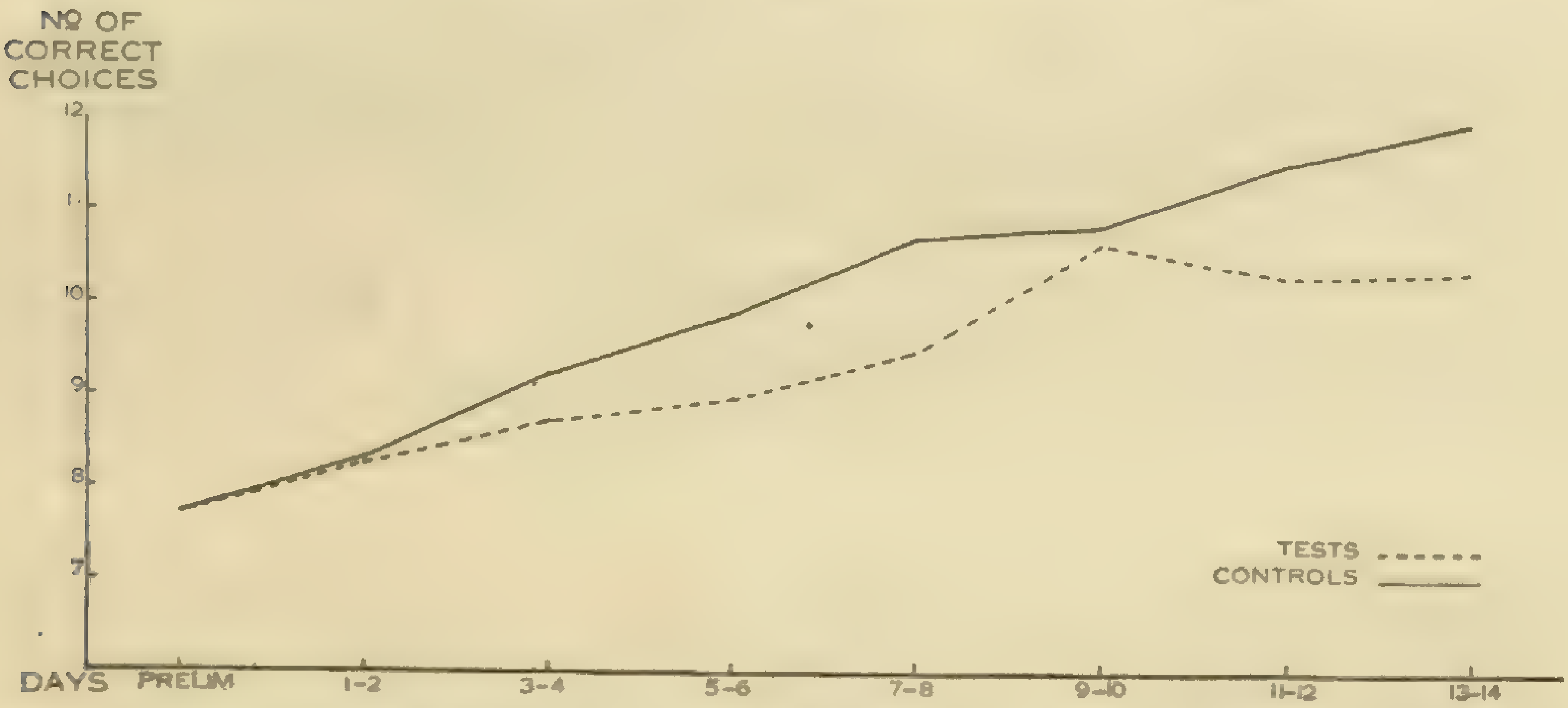

Fig. 3. Average numbers of correct first choices in the multiple choice apparatus, in the preliminary training and in the following pairs of days, when only those rats are included which made from 3–12 correct first choices in their preliminary training, *i.e.*, *eliminating* rats with strong tendencies either to choose or avoid the correct door, before regular training began. In this way the preliminary averages of the tests and controls are brought together and it becomes possible to compare the averages in the regular training.

Granting that the controls are adequate, the data on behavior indicate that a modification has been brought about by the alcohol; the generation showing the least absolute difference in maze-behavior is shown to be definitely modified when the tests are made on a multiple-choice apparatus.

Fertility

Compared with the difficulty of measuring the behavior tendencies of rats, the measure of fertility is very simple and definite. However, the great amount of time required by the behavior studies prevented the collection of many of the available data on the purely physiological side. As a result of this, instead of the long list of criteria of fertility that have been given by other authors, it is possible to give only two with any degree of accuracy and completeness. These are: the number of rats in a litter, and the number of litters. A more detailed report on the data leading to the following conclusions may be found elsewhere (MacDowell, '22*a*).

Size of Litters.—A general tendency for the litters of the test rats to be smaller than the controls persists in the summaries of all generations. The difference between the size of the litters from the original treated rats and the litters from the controls is equal to 10.5 per cent. of the size of the control litters. The treated offspring of the treated rats produced litters that were 10.3 per cent. smaller than the litters of their controls. It appears, therefore, that the treatment of the parents of the litters as well as the grandparents does not intensify the reduction in litter size found when only one generation was treated. The untreated offspring from treated rats gave litters that were 11.2 per cent. smaller than their controls, and the untreated offspring from untreated parents and treated grandparents gave litters that were 13.1 per cent. smaller than the controls (see Fig. 4). These differences in individual generations are based on too few cases to be significant when compared with their probable errors, but when the numbers are increased by taking all the generations together, the probable error is reduced so that the difference attains statistical significance (3.6 times its probable error). Litter size, then, gives a result not unlike that given by the behavior data: the tests are inferior in each generation, with no apparent relation to the proximity of the alcohol or the number of generations of treatment.

Number of Litters.—Given equal time, the treated pairs produced 0.72 litter per pair while the controls produced 2.07 litters per pair. This is a reduction of 64.8 ± 3.3 per cent. in the number of litters, and as it is 19.2 times its probable error, it is significant beyond all question. The test litters were slower in appearing than the controls. The treated rats from treated parents also gave fewer litters than their controls, but instead of a greater reduction than in the previous generation this second treated generation produced relatively more litters. The reduction was 35.4 ± 6.9 per cent. of the controls. Coming to the rats not directly treated, the untreated rats

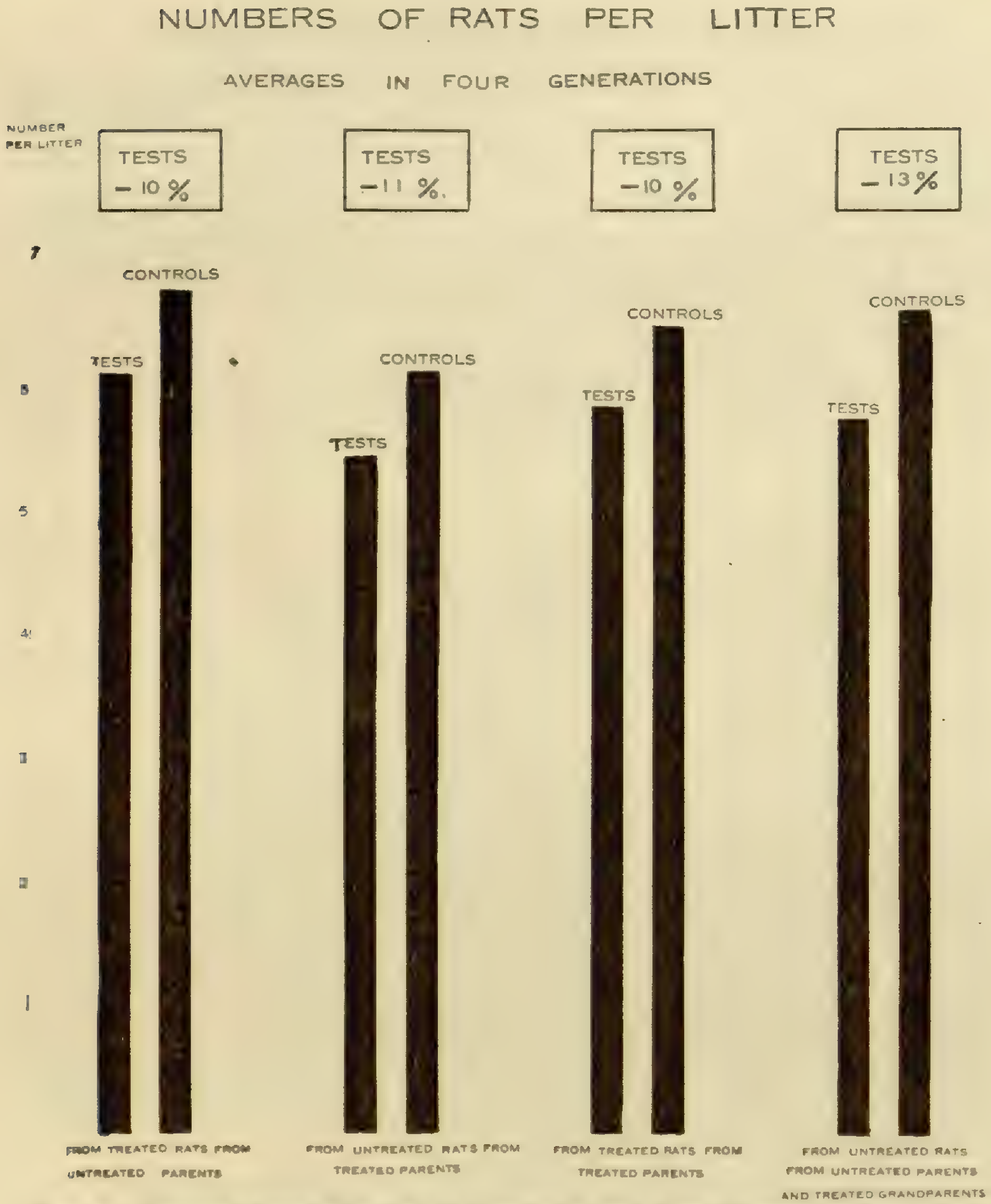

FIG. 4. Average litter size for the controls and tests bearing the relationships to the alcohol treatment indicated.

from treated parents gave 33.3 $\pm$ 8.2 per cent. *more* litters than their controls, and the untreated rats from untreated parents and treated grandparents produced 55.6 $\pm$ 8.4 per cent. *more* litters than their controls (see Fig. 5). All of these differences are, without doubt, statistically significant.

Discussion.—Two generations of treatment made less difference in number of litters than a single generation of treatment, and two untreated generations following

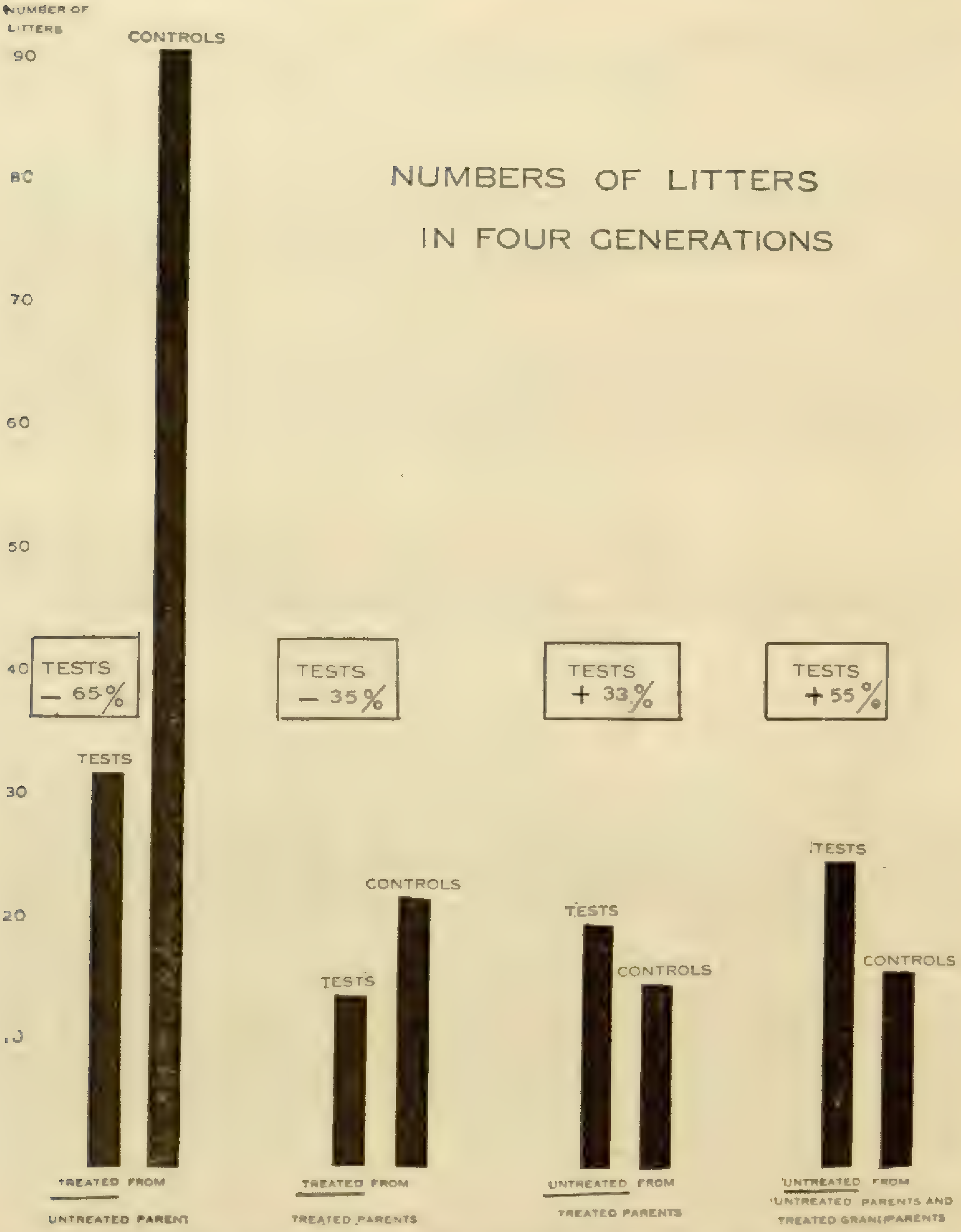

FIG. 5. Relative numbers of litters produced in equal periods by the test and control rats in different generations. Beginning at the left the test litters were produced by the following numbers of pairs: 44, 9, 10, 11; in each case the control litters have been given on the basis of equal numbers of pairs, although the actual numbers of control pairs involved were: 42, 12, 10, 13.

the treatment produced more litters than the controls. The number of litters is strongly reduced when the parents themselves are treated, but when the alcohol is more remote, the reduction vanishes and the untreated descendants of the treated rats produce *more* litters than their controls. To explain the reduction in the number of lit-

ters in the presence of alcohol along purely physiological lines would be a simple matter, but a genetic explanation appears to be required when it comes to the increase over the controls given by untreated descendants of treated animals. No general depression or stimulation will account for the continuation of small litters together with the increase in number of litters in the generations not given alcohol directly. It seems necessary to assume that there are genetic factors influencing the number of litters; alcohol prevents the reproduction of such females as carry factors working in the direction of lower reproductive capacity, so that the litters come alone from females carrying higher litter-producing capacity; the next generation will produce higher numbers of litters than the unselected controls, for the controls still carry all grades of fertility, while the tests lack the genetically lower grades. The treated offspring of treated rats produced fewer litters than their controls, but genetically they were superior, as shown by untreated offspring giving more litters than their controls; they were superior to the first generation, for, instead of a 65 per cent. reduction, they gave only a 35 per cent. reduction in the number of their litters. Whereas the immediate presence of alcohol reduces the number of litters, it acts to increase the number in the next generation; therefore alcohol may produce two results upon a single character in two generations. This could lead to much confusion were it not so easy to understand the first result as the cause of the second.

This selective action of alcohol will account for the results from the number of litters, but will not account for the uniform results given by litter size. If this is a correct statement of the situation, it indicates that the number of litters is influenced by genetic factors that are not identical with those influencing litter size. Although such a distinction between genetic bases for the numbers of litters and litter size has apparently not been made, it is not difficult to conceive, for litter size is largely dependent upon the number and constitution of the germ

cells liberated, while the somatic condition of the mother plays a part in determining whether or not a litter will be produced. The results from litter size agree strikingly, qualitatively and even quantitatively with those of Stockard and Papanicolaou from similar studies with guinea pigs; the results from the number of litters agree with Pearl's on fowl in so far as they may be interpreted by assuming a selective action of the alcohol working upon existing genetic differences. In the fowl the alcohol appears to select between germ cells; in the rats it appears to select between mothers of different physiological and genetic grades.

Weight

The data on weight (see MacDowell, '22*b*) form an extensive series consisting of weekly weighings of practically all the rats raised in the various generations herein described. Individual growth curves were plotted and from these the weights at six ages were taken for statistical study. This procedure was necessitated by the fact that all the rats were weighed on the same day each week, so that the rats were of different ages. The results are based primarily upon the males (see Table II), since the pregnancies of the females make their data less reliable. However, when the data from the females with arbitrary smoothing of the pregnancy peaks are summarized, the results so obtained support those given by the males. Each of the four strains shows that the treated rats grew more slowly than the controls. This is an influence shown by the population as a whole, although there are some individual treated males that remained as heavy as the heaviest controls. The untreated offspring of the treated rats tended to grow more rapidly than their controls. This result is not so clear as the opposite result in the preceding generation; the absolute differences are not so large and the strains do not show this in equal measure. Treated rats from treated parents barely differ at all from their controls. Very little can be concluded from the weights of the untreated offspring from untreated

TABLE II

THE WEIGHTS AT SUCCESSIVE AGES OF THE VARIOUS TYPES OF MALE TEST RATS COMPARED WITH THEIR RESPECTIVE CONTROLS.

Relation to Alcohol Treatment	Age in Days	Tests		Controls		Difference	D/P.E.
		Grams Averages	Nos.	Grams Averages	Nos.		
Treated from normal parents	40	70.43	70	72.95	64	+ 2.52 ± 1.40	1.8
	60	116.66	71	129.35	65	+12.69 ± 2.16	5.9
	90	149.63	61	176.63	55	+27.00 ± 3.16	8.5
	120	192.10	50	229.71	46	+37.61 ± 4.07	9.2
	150	215.39	38	257.97	39	+42.58 ± 4.81	8.8
	180	235.88	34	274.34	32	+38.46 ± 5.11	7.5
Treated from treated parents	40	83.86	15	81.81	16	− 2.05 ± 2.73	0.7
	60	128.00	15	131.81	16	+ 3.81 ± 3.81	1.0
	90	166.50	14	166.37	16	− 0.13 ± 4.96	0.0
	120	198.07	14	207.50	14	+ 9.43 ± 6.01	1.5
	150	228.33	12	236.50	14	+ 8.17 ± 6.48	1.2
	180	245.70	10	258.00	13	+12.30 ± 7.44	1.6
Untreated from treated parents	40	73.03	26	63.82	29	− 9.21 ± 2.37	3.8
	60	95.53	26	84.13	29	−11.40 ± 2.56	4.4
	90	127.26	26	117.79	29	− 9.47 ± 3.64	2.6
	120	195.26	26	182.14	27	−13.12 ± 5.13	2.5
	150	230.42	26	219.88	27	−10.54 ± 6.24	1.6
	180	249.00	25	238.85	27	−10.15 ± 7.61	1.3
Untreated from untreated parents and treated grandparents	40	75.90	10	80.57	7	+ 4.67 ± 5.08	0.9
	60	111.18	11	97.20	10	−13.98 ± 5.36	2.6
	90	131.54	11	125.90	10	− 5.64 ± 11.64	0.5
	120	191.63	11	171.30	10	−20.33 ± 16.66	1.2
	150	226.09	11	208.00	10	−18.09 ± 16.20	1.1
	180	235.54	11	230.20	10	− 5.34 ± 13.52	0.4

Plus signs indicate the controls heavier; *D*/PE.—difference divided by its probable error.

parents and treated grandparents. Two of the three strains represented in this generation show heavier averages for the tests and the third shows heavier averages for the controls; when all the strains together are considered (as in Table II), the test averages are higher at all ages.

This shows a marked similarity to the results from the number of litters; just as the offspring of the treated rats appear to be genetically superior to the controls in the matter of litter production, so they are found to be superior in the matter of weight, with the result that when they themselves are treated, the immediate reducing effect of the alcohol makes them about equal somatically to their controls, instead of growing markedly slower as did their parents. This likeness in results leads to a similar interpretation for the weight as for the number of litters: the alcohol has acted as a selective agent, eliminating germinal material that included factors for slower growth.

Discussion

In view of the premature termination of these experiments no discussion or interpretation can be justified other than by its possible influence upon future work.

The data on behavior and litter size taken alone may, if the controls are accepted as adequate, be considered to lead to the general interpretation of a direct and definite modification of the germinal material brought about by the alcohol treatment. On the other hand, the data on the number of litters and weight, when taken alone, agree in inviting the interpretation that the alcohol has acted as a selective agent upon germinal differences that were present in the germinal material of the original animals. One tendency pulls the race down, the other, by sacrificing the fullest reproductive expression of the treated individuals, tends to pull it up. The specific conditions found then are end-results that depend upon the interaction of different influences and do not measure directly the amount of influence exerted by the chemical.

Obviously, the situation is complicated, and equally obvious is the impossibility of proving the individual effects of two or more influences acting simultaneously. However, in this case the evidence favoring one supposition (that of selective elimination of germinal material) is very much more convincing than that favoring the supposition of germinal modification. So great, indeed, is this difference that the evidence of direct modification could easily be brushed aside and selective elimination be effectively championed as *the* effect of the alcohol, although even this involves two opposite results depending upon the proximity of the alcohol. But if a true statement of the situation is desired, the conflicting evidence must not be brushed aside.

If the germinal variability existing in the race is greater than the variability caused by the direct action of the alcohol upon the germinal material, the results actually obtained would be expected; that is, the effects of selective elimination would appear more striking in the end results. Since the reductions in litter size and in behavior stand in spite of an apparently much stronger racial improvement, these reductions give stronger support to the supposition that germinal modification is a second activity of the alcohol than is indicated by their magnitude.

The fact that so many different conclusions have been reached by different investigators from experiments with alcohol would in itself suggest very strongly that the action of this chemical upon animals is not simple and direct like the action of an acid upon a base, yet the general attitude toward the problem seems to have been that there should be a single answer, in one direction or the other, and that as soon as an investigator devises the perfect method, this answer will be disclosed. As long as such an attitude persists the alcohol problem will flounder about in the morass of futile and inconclusive papers. The moment chemistry, and later, experimental breeding, turned away from end results to the phenomena

behind them (elements or factors), new epochs were started in these sciences. The problem should not be to judge how bad are the results of alcohol, but rather to find through what channels alcohol may work. The final results will differ in different cases according to differently combined influences of various sorts, just as the same combination of chemicals will yield different results under different conditions, and the same combination of genetic factors will yield various somatic expressions; to know the *modus operandi* of alcohol is fundamental.

Conclusions

1. Beginning at the time of weaning, alcohol was administered to white rats every day, in sufficient quantities to cause complete anesthetization. This treatment appears to account for the following differences between the treated rats and their normal sibs:

The treated rats—(*a*) took more time running the maze.
(*b*) produced smaller litters.
(*c*) produced fewer litters.
(*d*) grew more slowly.

2. The treated offspring from the treated rats differed from their controls in the following ways:

The treated offspring—(*a*) tended to take more time in running the maze.
(*b*) produced smaller litters.
(*c*) produced somewhat fewer litters.
(*d*) grew at a very slightly lower rate.

3. The untreated offspring from the treated rats differed from their controls in the following ways:

The untreated offspring—(*a*) took a very little longer in running the maze.
(*b*) produced smaller litters.
(*c*) produced more litters.
(*d*) were heavier.

4. The untreated offspring in the second generation from alcohol treatment differed from their controls in the following ways:

The second generation of untreated offspring—

(*a*) took more time in running the maze.
(*b*) produced smaller litters.
(*c*) produced more litters.
(*d*) were somewhat heavier.

5. From these results it is concluded that the action of alcohol is complicated; that it works in two or more different ways. The data on behavior and litter size suggest that the alcohol may modify germinal material directly. The data on the number of litters and growth indicate that the direct effect of alcohol upon these characters is in one direction and that its indirect effect is in the opposite direction; this may be interpreted by the assumption of a selective rôle played by the alcohol. It is urged that the alcohol problem can be settled biologically only when, instead of generalizing from the quality of specific end results, we deal with the channels through which alcohol may work.

Cold Spring Harbor,
February, 1922.

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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE. IV. DATA ON THE INFLUENCE OF DENSITY OF POPULATION ON DURATION OF LIFE IN DROSOPHILA[1]

PROFESSOR RAYMOND PEARL AND SYLVIA L. PARKER

I

FAIRLY early in our experimental work on duration of life in *Drosophila* it became apparent to us that the number of flies per bottle, or, since the bottles used are of uniform size, the density of population, had some influence on the mean duration of life of the flies, when other environmental conditions are constant. Such a relationship might reasonably be expected *a priori,* from what is known of the influence of this factor on human death rates, commonly expressed as Farr's Law (*cf*. Farr, W. (35), Brownlee, J. (36, 37)), and on other biological functions, such as growth (Semper, K. (38), Bilski, F. (39)), resistance to poisons (Drzwina and Bohn (40)), rate of reproduction (Pearl and Surface (41), Pearl and Parker (42)), etc. As soon as it was recognized that this variable, density of population, might influence our experimental results with *Drosophila,* care was taken in setting up experiments to make this a constant in each case. At the same time the records of the earlier work were carefully re-examined to determine what part this variable may have played in the results. Happily it was found that in none of our work so far published upon the duration of life in *Drosophila* had density of population varied enough to have any appreciable effect upon the results or conclusions.

As was recently pointed out by Pearl and Parker (42), however, "there can be no question that this whole matter of influence of density of population, in all senses, upon biological phenomena, deserves a great deal more

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University. No. 63.

investigation than it has had. The indications all are that it is the most important and significant element in the biological, as distinguished from the physical, environment of organisms.'' In pursuance of this idea we desire to present in this paper our accumulated *statistical* data on the influence of density of population upon duration of life in *Drosophila.* This material is to be regarded as preliminary rather than final. For reasons which will appear as we proceed, we are inclined to withhold final conclusions as to the exact form of the regression of duration of life upon density until we have completed an extensive *ad hoc* experimental investigation of the problem. This experimental work is now in progress and we hope to be able to report upon it in full in the course of the next year. In the meantime we have an impressive body of statistical data gathered from the control groups of other experiments which it seems desirable to discuss now in a preliminary way.

II

The data of this study are derived from the normal control groups of various experiments on duration of life which we have carried out with *Drosophila,* according to the technique described by Pearl and Parker (27). All of the determinations of duration of life recorded in the tables of this paper were made under constant conditions of temperature (25° C.), food, etc., as described in the paper referred to. We have divided the material for the purposes of the present study into three groups by stocks (*cf.* Pearl and Parker (27)), *viz.:* (*a*) wild type flies, including our Old Falmouth, New Falmouth, and Eagle Point stocks, (*b*) Sepia, and (*c*) Quintuple.

Throughout this paper density of population is taken as the *initial density* (number of flies per bottle) in the small bottles used in testing duration of life. Thus a density of 22 means that 22 flies started in this particular bottle. As time went on the number was diminished by deaths until finally none was left. One of course might use as the variable mean density over the whole life of a

bottle, but a little thought will show that this would be an erroneous procedure when one is dealing with duration of life as the second variable, because *mean* density bears a direct and implicit functional relation to mean duration of life of the flies in the bottle. We shall be on a clearer footing to take *initial* density as the variable. Since the cubical content of the bottles is constant throughout, there is no necessity of reckoning density per c.c. The number of flies per bottle can be taken as the measure of density, and a good deal of useless computation saved.

We are indebted to Dr. John Rice Miner for aid in the computations.

III

Table I presents the data for the correlation of duration of life with density of population for the wild type flies. The material is in the usual form of a correlation table.

An examination of this surface suggests at once that the regression is probably non-linear. Owing to the manner in which the material was obtained (by compilation of the control series of a number of different experiments) it results that the different arrays have rather highly different total frequencies. The number of flies per bottle was in no way artificially selected or predetermined in this material. Instead it was determined solely by the aggregate fertility of the mating bottles furnishing the material for each particular experiment. As has been explained in the first of these Studies (Pearl and Parker (27)), the routine procedure in our experiments is to put into one bottle for duration of life test all the flies emerging as imagoes at the same time (*i.e.*, usually on the same day). It therefore would result that if the hatch was particularly good on some day, there might be as many as 90 flies in the duration of life bottle initially. On the other hand, there might be only 2 flies, because only that number emerged on that particular day.

Even in spite of the differences in the frequencies of

TABLE I

CORRELATION SURFACE FOR THE VARIABLES (*a*) DURATION OF LIFE, AND (*b*) INITIAL DENSITY OF POPULATION. WILD STOCKS OF DROSOPHILA

Age at Death	Number of Flies in Bottle																								
	1–	5–	9–	13–	17–	21–	25–	29–	33–	37–	41–	45–	49–	53–	57–	61–	65–	69–	73–	77–	81–	85–	89–	93–	Total
1–......	15	41	35	29	35	45	37	3	27	4	10	28	20	....	12	19	....	12	....	....	....	2	....	16	390
7–......	38	49	47	48	39	38	30	20	22	6	6	8	24	4	13	11	1	17	13	2	....	....	....	43	479
13–......	24	55	44	45	59	35	39	20	28	3	3	12	14	23	14	19	1	8	10	5	1	2	....	20	484
19–......	19	53	57	30	64	37	38	33	24	17	51	30	32	14	17	33	7	5	46	13	10	22	....	47	699
25–......	31	75	59	45	70	42	79	21	31	22	20	20	32	7	17	20	15	7	40	3	15	21	....	5	697
31	19	87	88	55	76	90	78	47	44	10	47	31	33	5	13	68	11	23	50	13	16	52	....	4	960
37	14	72	108	84	118	94	80	77	45	19	31	36	60	3	38	70	16	4	20	11	14	27	....	8	1,049
43–......	21	73	126	86	161	83	80	39	53	16	32	66	67	....	23	18	46	32	33	5	17	2	....	19	1,098
49–......	19	84	110	109	120	124	117	43	71	20	44	71	70	....	14	35	23	33	7	11	7	12	....	2	1,146
55–......	10	75	85	102	102	71	109	47	37	38	56	35	37	....	15	38	6	16	1	4	....	17	....	9	910
61–......	5	38	61	96	97	55	70	50	39	35	42	55	49	....	11	16	3	18	2	11	1	7	....	13	774
67–......	1	25	36	54	66	41	75	19	13	17	18	44	31	....	11	7	3	3	....	1	1	5	....	....	471
73–......	....	9	11	33	33	34	30	19	8	13	13	13	15	....	21	9	1	15	....	1	....	....	....	....	278
79–......	....	5	4	20	17	13	16	10	4	5	4	10	11	....	2	6	....	6	....	....	....	....	....	....	133
85–......	1	2	6	8	3	8	9	5	3	....	2	2	2	....	6	3	....	4	....	....	....	....	....	....	64
91–......	....	....	....	3	....	....	5	3	2	1	1	2	1	....	5	....	....	5	....	....	....	....	....	....	28
97–......	....	....	....	....	....	1	....	....	....	....	....	....	....	....	1	2	....	1	....	....	....	....	....	....	5
103–......	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....	1
109–......	....	....	....	....	....	1	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	....	1
Total....	217	743	877	847	1,060	812	892	456	451	226	380	463	498	56	234	374	133	209	222	80	82	169	....	186	9,667

the several arrays, it still seems probable from mere inspection of the general surface that the regression is non-linear. This idea is strengthened by examination of the regression line itself, shown in Fig. 1.

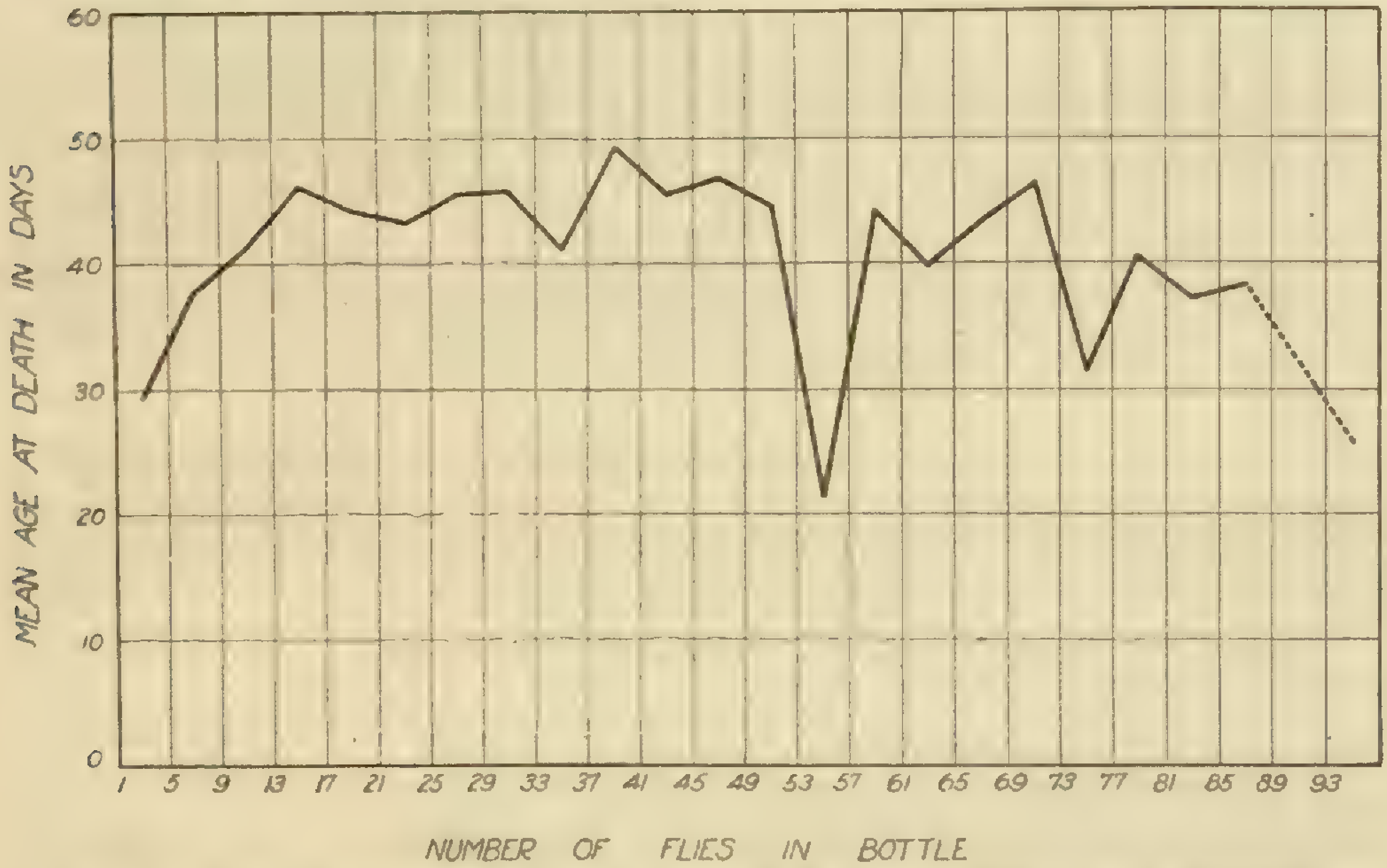

FIG. 1. Mean duration of life of *Drosophila* for different initial densities of population. Wild stocks.

It is seen from this diagram that, neglecting the great dip of the line at density 55 which is consequent upon a very small array with large probable error, the general sweep of the curve indicates an optimum density (greatest mean duration of life) in the general region of 35 to 45 flies per bottle, with a decline on either side of that point, but falling lower on the side of high densities than on that of low.

From this table we have the following constants:

$$r = -.0511 \pm .0068,$$
$$\eta = \quad .2443 \pm .0064.$$

There can be no question that the regression is non-linear. Blakeman's (43) criterion has the following value:

$$\zeta = \quad .0571 \pm .0031.$$

It must therefore be concluded that the regression is significantly skew.

The correlation between duration of life and density of population in the case of the Sepia stock is shown in Table II.

TABLE II

Correlation Surface for the Variables (*a*) Duration of Life, and (*b*) Initial Density of Population. Sepia Stock

Age at Death	Number of Flies in Bottle																					
	1–	5–	9–	13–	17–	21–	25–	29–	33–	37–	41–	45–	49–	53–	57–	61–	65–	69–	73–	77–	81–	Total
1–..........	7	29	27	23	13	15	....	1	1	....	2	....	....	....	....	....	....	....	4	1	37	160
7–..........	10	18	27	27	11	27	2	16	....	5	3	....	....	....	4	....	....	....	3	9	6	168
13–..........	5	21	23	23	6	33	12	18	1	8	4	....	....	....	8	....	....	....	5	46	1	214
19–..........	16	27	8	35	9	21	12	21	9	35	8	6	10	....	6	....	....	....	21	17	2	263
25–..........	7	34	5	20	8	30	17	21	14	26	9	30	15	....	18	....	....	....	38	2	7	301
31–..........	13	28	40	20	9	20	9	13	8	24	22	4	13	....	14	....	....	....	41	....	....	278
37–..........	4	28	30	40	15	33	15	7	1	15	11	4	3	....	6	....	....	....	8	2	2	224
43–..........	9	26	38	35	23	37	12	13	1	24	30	1	1	....	2	....	....	....	27	....	15	294
49–..........	10	27	36	24	17	19	1	6	....	9	16	1	4	....	1	....	....	....	3	....	11	185
55–..........	5	18	16	17	19	18	....	2	....	2	8	....	1	....	1	....	....	....	1	....	....	108
61–..........	3	2	15	10	9	6	1	6	....	3	7	....	4	....	....	....	....	....	1	....	....	67
67–..........	....	9	6	4	5	3	....	1	....	....	7	....	....	....	....	....	....	....	....	....	....	35
73–..........	....	....	2	2	2	1	....	....	....	1	2	....	....	....	....	....	....	....	....	....	....	10
79–..........	1	....	....	2	....	1	....	....	....	....	2	....	....	....	....	....	....	....	....	....	....	6
Total........	90	267	273	282	146	264	81	125	35	152	131	46	51	....	60	....	....	....	152	77	81	2,313

Here again there are a number of small arrays and gaps towards the right-hand side of the table, due as before to the method by which the material was got.

The regression of duration of life upon density is shown graphically in Fig. 2.

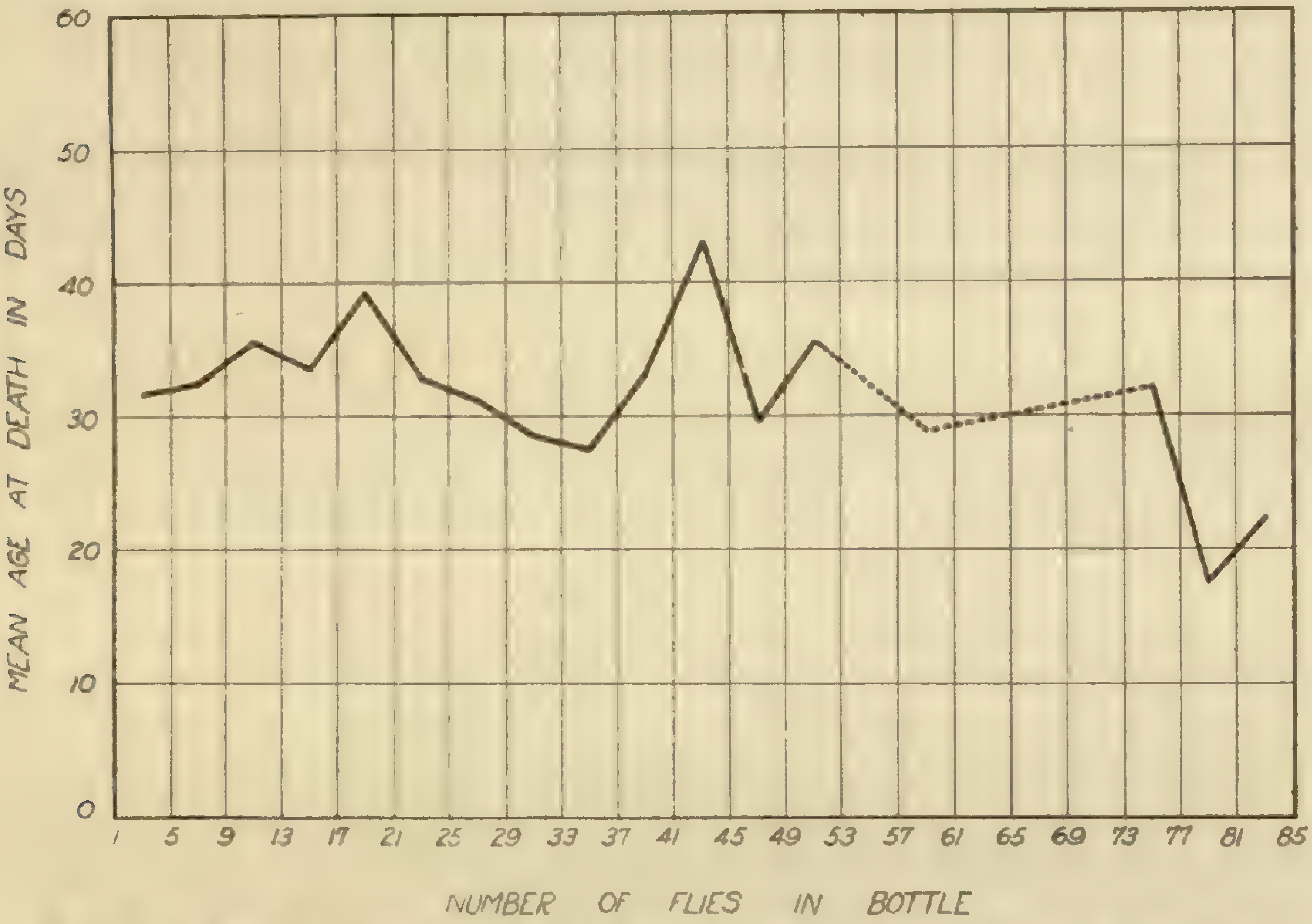

FIG. 2. Mean duration of life of *Drosophila* for different initial densities of population. Sepia stock.

It is apparent from inspection here as before that the regression is not clearly linear, but rather indicates an optimum density in the region of 35 to 45 flies per bottle, with a diminished expectation of life at both lower and higher densities. The constants are

$$r = -.132 \pm .014,$$
$$\eta = .283 \pm .013,$$
$$\zeta = .0629 \pm .0066.$$

The criterion of linearity is nearly 10 times its probable error, and we may therefore conclude for the Sepia stock, as for the wild stocks, that statistically the regression of duration of life upon density of population is significantly skew.

The data for the short-lived Quintuple stock are given in Table III.

Owing to the fact that the Quintuple stock is characterized by low fertility, as well as short duration of life,

TABLE III

CORRELATION SURFACE FOR THE VARIABLES (*a*) DURATION OF LIFE, AND (*b*) INITIAL DENSITY OF POPULATION. QUINTUPLE STOCK

Age at Death	Number of Flies in Bottle													
	1–	5–	9–	13–	17–	21–	25–	29–	33–	37–	41–	45–	49–	53–	Total
1–	22	18	17	15	9	7	15	1	...	...	...	...	...	4	108
4–	21	33	19	31	4	3	9	11	...	...	...	...	...	15	146
7–	26	70	50	50	13	13	20	4	...	...	...	...	...	17	263
10–	22	38	47	28	19	12	10	...	...	...	...	...	...	7	183
13–	15	24	28	29	8	13	12	6	...	...	...	...	...	3	138
16–	8	21	20	19	6	7	4	4	...	...	...	...	...	...	89
19–	11	14	11	12	4	3	1	...	...	...	...	...	...	1	57
22–	5	13	12	4	6	2	1	...	...	...	...	...	...	1	44
25–	2	5	13	7	3	3	2	...	...	...	...	...	...	...	35
28–	....	6	3	3	2	2	1	2	...	...	...	...	...	2	21
31–	....	6	3	1	...	3	1	...	...	...	...	...	...	1	15
34–	2	3	....	3	...	...	...	...	...	...	...	...	...	...	8
37–	1	4	2	....	...	1	...	...	...	...	...	...	...	1	9
40–	1	3	2	....	...	...	2	1	...	...	...	...	...	1	10
43–	3	2	2	....	...	1	...	...	...	...	...	...	...	...	8
46–	....	....	....	....	...	...	...	...	...	...	...	...	...	...	......
49–	....	1	1	....	...	...	1	...	...	...	...	...	...	...	3
Total	139	261	230	202	74	70	79	29	...	...	...	...	...	53	1,137

this table is less extensive in either direction than the others.

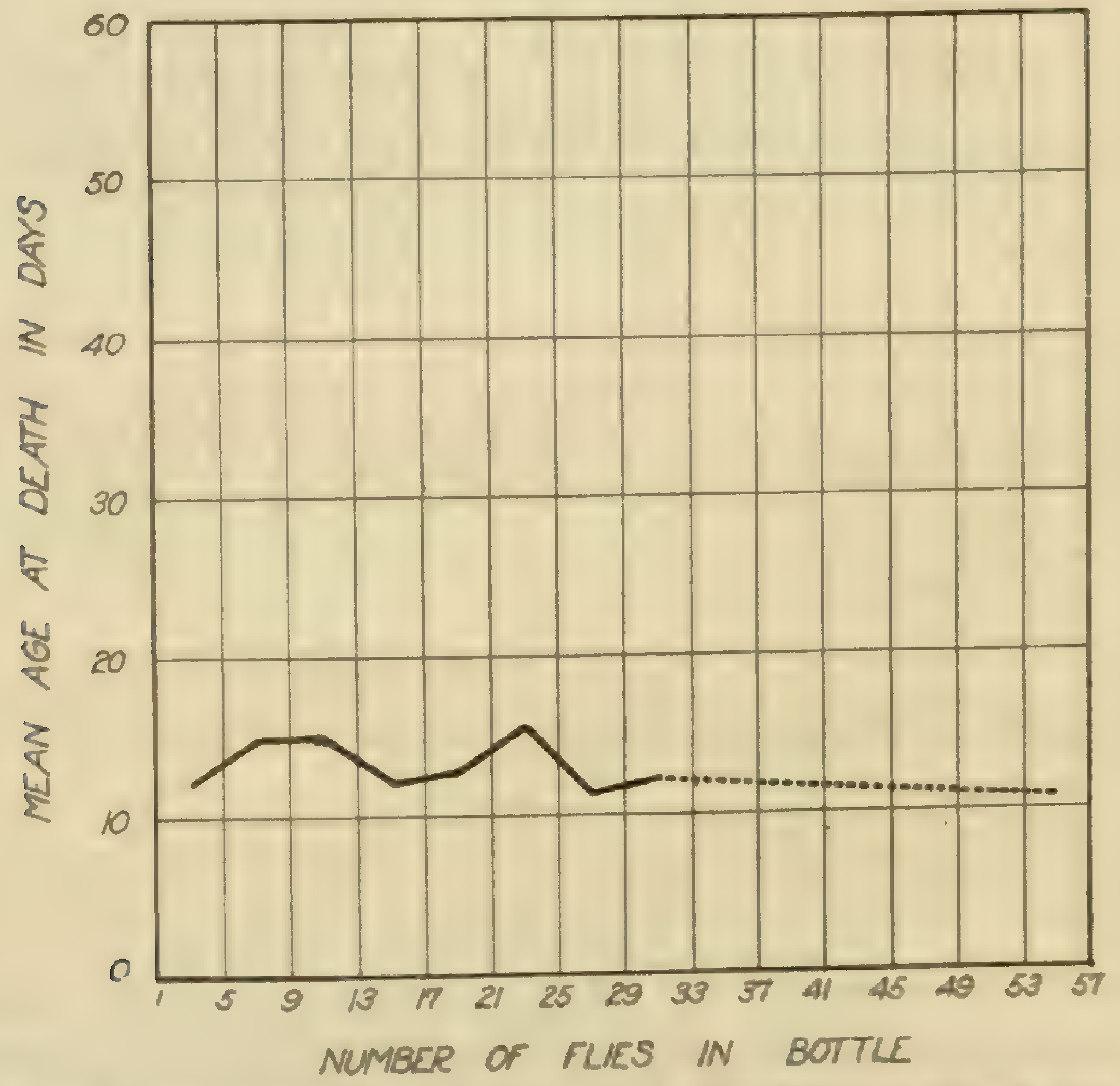

FIG. 3. Mean duration of life of *Drosophila* for different initial densities of population. Quintuple stock.

The observed regression line is shown in Fig. 3.

Here the regression appears at once to be substantially linear, and is proved to be by the analytical constants, which are as follows:

$$r = -.057 \pm .020,$$
$$\eta = .120 \pm .020,$$
$$\zeta = .011 \pm .004.$$

The criterion ζ is less than 3 times its probable error and cannot be regarded as significant.

IV

Putting all the data together, we have here indisputable evidence that the density of population is a significant factor in influencing the duration of life (or death-rate) in *Drosophila*. The correlation ratio η is certainly significant in the case of all three stocks. Its lower value in the case of the Quintuple stock is almost certainly due to the fact that in the Quintuple experience there is not a sufficiently extensive representation of densities. If the other two tables were to be cut off at the density array where the Quintuple is, they also would show a much lower association between the two variables. So, then, the *general portion* of Farr's Law which affirms that death-rate is some function of density of population receives experimental confirmation in a widely different form of life.

When one comes, however, to the precise form discovered by Farr (35) and confirmed by Brownlee (36, 37), the case is not so clear. We do not care to enter upon any detailed discussion of the point now, because we do not care to draw any conclusions as to the true form of the skew regressions observed till we have some additional experimental results in hand. Provisionally, however, it may be said that the indications are that in *Drosophila* something like the following relations hold: (*a*) the lowest density is not the optimum; (*b*) the mean duration of life tends to increase with increasing density up to a certain point which is optimum; (*c*) after the

optimum region has been reached, increasing density is associated with diminished duration of life, which presently falls below the lowest figure found with densities below the optimum. These conclusions must for the present be held as tentative.

V

In this paper data as to total duration of imaginal life of 13,117 individuals of *Drosophila* are presented in relation to the density of population. It is definitely shown in the case of Wild, Sepia and Quintuple stocks that there is a significant correlation between these variables. The regression of duration of life upon density appears to be significantly skew in the case of Wild and Sepia stocks. The precise form of the regression and theoretical questions connected therewith are left for discussion in a later paper upon the basis of more extensive material.

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NOTES ON THE HYBRIDS BETWEEN THE CANARY AND TWO AMERICAN FINCHES

O. E. PLATH

Massachusetts Institute of Technology, Cambridge, Mass.

Perhaps no animal has been so often crossed with other species, and even genera, as the domesticated canary (*Serinus canarius*). Darwin (1885, I, p. 311) speaks of "nine or ten" such crosses, but many more have undoubtedly been made. The hybrids resulting from these crosses are usually, if not always, infertile, and hence are popularly known as "mules." In almost all of these crosses the domesticated canary serves as the female and the wild finch as the male, but bird fanciers occasionally succeed in making the reverse cross. The wild species which is most commonly used for this "mule breeding" is the European goldfinch, *Carduelis carduelis* Linnæus.[1] This fringillid is one of the handsomest finches in existence, the plumage of the adults of both sexes being made up of a beautiful combination of black, red, white, yellow, and brown patches. The hybrids which result when a yellow, or nearly yellow, canary is crossed with this finch are chiefly interesting for two reasons: (1) because they exhibit an apparently endless chain of variability in coloration, and (2) because their plumage, if dark, is conspicuously streaked, a character which is lacking (as far as external appearance is concerned) in both the yellow canary and the European goldfinch.

Concerning the first of these two points valuable data have been published by Bechstein (1795), Hünefeld (1864), Blakston (1880?), Klatt (1901), Davenport

[1] According to Chapman (1916, p. 383), this finch was introduced into the United States at Hoboken, N. J. (in 1878), and Boston, and probably still is a resident near both of these places.

(1908), and Galloway (1909). According to these authors, the hybrids between the yellow canary and the European goldfinch may be: (*a*) completely dark, (*b*) mottled (spotted), exhibiting an apparently endless variation in color pattern, or (*c*) entirely white or yellow (very rarely).[2]

The streaking in the dark plumage of canary-European goldfinch hybrids has been variously explained as: (*a*) "derived from the original wild canary" (Darwin, 1885, II, p. 15); (*b*) as reversion to the Serin finch, *Serinus hortulanus* Koch (Klatt, 1901, p. 508); and (*c*) as resulting from the latent streaking (visible in the "green" variety of the domesticated canary) factor of the yellow canary, plus the color factor of the European goldfinch (Davenport, 1908, p. 20).

In 1914 the writer made several attempts to cross the domesticated canary with some of our native American finches, and some of the latter among themselves, since such crosses, if made, seem to have never been recorded. None of these experiments were successful. The work was again taken up in the fall of 1918, and this second attempt yielded several hybrids in 1919 and 1920. For these latter experiments the writer had at his disposal 22 wild finches belonging to the following species: Arkansas goldfinch (*Astragalinus psaltria hesperophilus* Oberholser), willow goldfinch (*Astragalinus tristis salicamans* [Grinnell]),[3] California linnet (*Carpodacus mexicanus frontalis* [Say]), and California purple finch (*Carpodacus purpureus californicus* Baird). Of these 22 wild finches, 5 were reared from eggs placed under

[2] Galloway (1909, p. 4), who has probably reared more canary-finch hybrids than any other breeder, reports the following proportions of self-colored to variegated (mottled) individuals in the case of canary-European gold-finch hybrids: (1) dark plumage (with no white or clear feathers), 172; (2) slightly variegated (a few small white or clear spots in an otherwise dark plumage), 74; (3) variegated (1/4 to 1/2 clear), 75; (4) lightly variegated (1/2 clear to small ticks of dark in an otherwise clear plumage), 19; and (5) completely clear (total absence of dark feathers), 0.

[3] A western sub-species of the American goldfinch (*Astragalinus tristis tristis* Linnæus), popularly known as the "wild canary."

canary females and the remaining 17 were trapped shortly before the breeding season. It is chiefly due to this second fact that the number of hybrids obtained was not larger. All of the experiments were carried out in separate breeding cages. The matings which yielded results were the following:

TABLE I

Cross No.	Year	♀ × ♂	No. of Offspring
1........	1919	Yellow canary × California linnet	3
2........	1920	Yellow canary[4] × Willow goldfinch	5
3........	1920	Willow goldfinch × Arkansas goldfinch	4

The four hybrids resulting from cross No. 3 (willow goldfinch ♀ × Arkansas goldfinch ♂) died a few days after hatching, and the female could not be induced to breed for a second time. These hybrids differed from ordinary newly-hatched finches and from the eight hybrids obtained from crosses No. 1 and No. 2 in having exceedingly large abdomens, a condition which was probably due to the fact that a large quantity of yolk had not been assimilated.

Cross No. 1 (yellow canary ♀ × California linnet ♂) yielded three hybrids, one of which was accidentally killed when nine days old. During the same summer (1919) Mrs. L. V. Irelan of Berkeley, California, likewise succeeded in rearing a brood (2 males and 2 females) of canary-California linnet hybrids[5] which the writer was able to compare with his own.

Before going into detail regarding the coloration of these canary-California linnet hybrids, it seems desirable to refer briefly to the plumage color of the paternal species, the California linnet. Both sexes of this finch are grayish-brown in color, but, when about three months old, the male turns rose pink, orange red, or scarlet about

[4] The same female which was used in cross No. 1.

[5] In this case the mother was also completely yellow.

the head, neck, breast and rump. These colors increase in extent and brilliancy with each molt. Males reared and kept in captivity never develop anything but a yellowish-buff color in these regions, and if a mature wild male is confined, its red color, during the molt, likewise becomes yellowish-buff. Both adults and young are conspicuously streaked, especially the latter.

The six[6] canary-California linnet hybrids were all completely dark (self-colored) until the first molt (fall 1919), and closely resembled young California linnets, but their plumage was less intensely dark than that of the latter. During the fall molt of 1919 all of the hybrids became slightly "washed" (tinged) with yellow where the California linnet ♂ is red (or yellowish-buff). This yellow tinge was more conspicuous in the males than in the females and became somewhat more pronounced during the fall molt of 1920.

All six canary-California linnet hybrids are streaked, like the paternal and the "green" variety of the maternal species. As regards size and shape, they differ very little from the parents, both of which are similar in these respects. Their notes are intermediate in timbre between those of the two parental species, the males having a more powerful song than the canary.

In the spring of 1920 the writer paired two of these canary-California linnet hybrids. Both showed an ardent desire to breed and the female exhibited considerable skill in nest building. The first egg was laid on May 6, and several days later a second (May 10). Both of these eggs were only about half the size of canary or California linnet eggs[7] and were dark-blue in color, and not speckled, while those of both parental species are bluish-white and speckled. Both eggs were placed under canary females, but proved to be infertile. The male

[6] The hybrid which was accidentally killed was identical in coloration with these six.

[7] This corroborates similar observations by Bechstein (1795, IV, p. 469) and Blakston (1880?, p. 265), both of whom compare the eggs of canary-finch hybrids with peas.

used in this experiment was also mated with a yellow canary, but, despite much treading, all eggs were clear.

From cross No. 2 (yellow canary ♀ × willow goldfinch ♂) five[8] hybrids were obtained. A few years before, Dr. H. C. Bryant of the California Fish and Game Commission also succeeded in rearing a canary-willow goldfinch hybrid, concerning which he has been kind enough to furnish the writer with complete information.

Before considering the plumage color of these canary-willow goldfinch hybrids, it seems again desirable to sketch briefly that of the wild finch. Both young and adults of the willow goldfinch are chiefly olive-brown and black in color, but the sexually mature male turns canary-yellow during the summer, with the exception of the wings, tail and a small patch on the head, which remain black. Neither young nor adults show any streaking.[9]

The three canary-willow goldfinch hybrids reared by the writer are (January 6th, 1921) colored as follows: No. 1, completely dark (self-colored); No. 2, likewise, except for a few yellow feathers near the left eye; No. 3, dark, with a yellow band, about 5 mm. in width, running across the head; No. 4 (reared by Dr. Bryant),[10] dark, with some white feathers on the tail. All of the hybrids reared by the writer are conspicuously streaked, which, according to Dr. Bryant, was also true of hybrid No. 4.

As regards size and shape, the writer's canary-willow goldfinch hybrids closely resemble the canary (this was also true of hybrid No. 4), especially in shape of beak and length of tail, in which respects there is a considerable difference between the two parental species. As in

8 Two of these died shortly after hatching and hence furnished no reliable data as regards coloration.

9 This is also true of the remaining North American members of the genus Astragalinus, the Arkansas and the Lawrence goldfinch (*Astragalinus lawrencei* Cassin), except that in the case of the latter, the lower parts of the young are indistinctly streaked (cf. Bailey, 1912, pp. 322, 323).

10 The canary mother of this hybrid was also completely yellow.

the case of cross No. 1 (yellow canary ♀ × California linnet ♂), the notes of the hybrids are intermediate in timbre between those of the parents.

We now come to the question as to how these hybrids compare with other canary-finch hybrids, and in how far they conform with Mendel's laws of inheritance. It will be noticed that in the case of the canary-California linnet hybrids, as in many mammalian crosses, dark color is completely dominant over light color, but the number of offspring (7) is too small to warrant the conclusion that this will always prove to be the case. On the other hand, as regards the canary-willow goldfinch hybrids, there is no complete dominance of one color, the hybrids in this case showing a similar variability to that of canary-European goldfinch hybrids.

Davenport (1908, p. 23) believes that the variability in plumage color of canary-finch hybrids is entirely due to the "mottling factor" of the yellow canary. He says (p. 23):

> It [the yellow canary] carries a mottling factor. Consequently when the yellow canary is crossed with a pigmented canary or with a finch the hybrids are mottled.

In support of this hypothesis he makes the following statement:

> That it is the yellow canary which contains the mottling factor and is the source of the variability of the hybrids is shown by the fact that (1) hybrids with the green canary do not vary in this fashion, and (2) hybrids between any two species of finches—of which many are bred by fanciers—are "cast in one mold."

As regards the first of these two points, it may be said that one should not expect canary-finch hybrids from a "green" (self-colored) canary to show yellow markings as frequently as when a yellow canary is used. In regard to the second point, Davenport (1908) seems to have overlooked the fact that Blakston (1880?), on whose authority this statement was probably based, states only (p. 274) that all bullfinch-goldfinch "mules" are "cast in one mould." In fact one of Blakston's (1880?) re-

marks clearly indicates that this is not true of the hybrids between all species of finches, for on the next page (275) he makes the following statement concerning the "much more common" greenfinch-goldfinch hybrid:

> It is not a very pretty bird, . . . partaking to a considerable extent of its [the greenfinch's] dull colour, though occasionally a more brilliant example than usual, having a good deal of the Goldfinch character about it, appears on the stage.

Davenport's (1908) conclusion therefore does not seem to be very well founded.

Results published by Galloway (1909) since the appearance of Davenport's (1908) paper seem to throw some light on this question. As already stated, this author (Galloway) obtained 172 dark (self-colored) to 168 variegated (mottled) offspring from his canary-European goldfinch (*Carduelis carduelis*) crosses. However, when he used the siskin (*Carduelis spinus*), a closely related but darker species, he obtained nearly three times as many (36 to 13) self-colored as mottled individuals, that is, almost a 3 to 1, instead of a 1 to 1 ratio. These results, supported by those set forth in this paper, suggest that the frequency of mottling in canary-finch hybrids is not solely due to the yellow canary,[11] but probably also depends on the coloration of the wild finch.

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[11] A similar problem exists in regard to the mottled seed-coat of the F_1 of certain pigmented-white bean crosses. Shull (1907) suggested that it is the white, and not the pigmented bean to which the mottling is due. However, Tschermak (1904, 1912) has shown that in some cases it is the pigmented bean which is the source of the mottling, a view which was later accepted by Shull (1908, pp. 437–439).

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COEFFICIENTS OF INBREEDING AND RELATIONSHIP

DR. SEWALL WRIGHT

Bureau of Animal Industry, United States Department of Agriculture

In the breeding of domestic animals consanguineous matings are frequently made. Occasionally matings are made between very close relatives—sire and daughter, brother and sister, etc.—but as a rule such close inbreeding is avoided and there is instead an attempt to concentrate the blood of some noteworthy individual by what is known as line breeding. No regular system of mating such as might be followed with laboratory animals is practicable as a rule.

The importance of having a coefficient by means of which the degree of inbreeding may be expressed has been brought out by Pearl[1] in a number of papers published between 1913 and 1917. His coefficient is based on the smaller number of ancestors in each generation back of an inbred individual, as compared with the maximum possible number. A separate coefficient is obtained for each generation by the formula

$$Z_n = 100\left(1 - \frac{q_{n+1}}{p_{n+1}}\right) = 100\left(1 - \frac{q_{n+1}}{2^{n+1}}\right)$$

where $q_{n+1}/2^{n+1}$ is the ratio of actual to maximum possible ancestors in the $n + 1$st generation. By finding the ratio of a summation of these coefficients to a similar summation for the maximum possible inbreeding in higher animals, *viz.*, brother-sister mating, he obtains a single coefficient for the whole pedigree.

This coefficient has the defect, as Pearl himself pointed

[1] American Naturalist, 1917, 51: 545–559; 51: 636–639.

out, that it may come out the same for systems of breeding which we know are radically different as far as the effects of inbreeding are concerned. For example, in the continuous mating of double first cousins, an individual has two parents, four grandparents, four great grandparents and four in every generation, back to the beginning of the system. Exactly the same is true of an individual produced by crossing different lines, in each of which brother-sister mating has been followed. Yet in the first the individual will be homozygous in all factors if the system has been in progress sufficiently long; in the second he will be heterozygous in a maximum number of respects.

In order to overcome this objection Pearl has devised a partial inbreeding index which is intended to express the percentage of the inbreeding which is due to relationship between the sire and dam, inbreeding being measured as above described. A coefficient of relationship is used in this connection. These coefficients have been discussed by Ellinger[2] who suggests certain alterations and extensions by means of which the total inbreeding coefficient, a total relationship coefficient and a total relationship-inbreeding index for a given pedigree can be compared on the same scale.

An inbreeding coefficient to be of most value should measure as directly as possible the effects to be expected on the average from the system of mating in the given pedigree.

There are two classes of effects which are ascribed to inbreeding: First, a decline in all elements of vigor, as weight, fertility, vitality, etc., and second, an increase in uniformity within the inbred stock, correlated with which is an increase in prepotency in outside crosses. Both of these kinds of effects have ample experimental support as average (not necessarily unavoidable) consequences of inbreeding. The best explanation of the decrease in vigor is dependent on the view that Mendelian

[2] American Naturalist, 1920, 54: 540–545.

factors unfavorable to vigor in any respect are more frequently recessive than dominant, a situation which is the logical consequence of the two propositions that mutations are more likely to injure than improve the complex adjustments within an organism and that injurious dominant mutations will be relatively promptly weeded out, leaving the recessive ones to accumulate, especially if they happen to be linked with favorable dominant factors. On this view it may readily be shown that the decrease in vigor on starting inbreeding in a previously random-bred stock should be directly proportional to the increase in the percentage of homozygosis. Numerous experiments with plants and lower animals are in harmony with this view. Extensive experiments with guinea-pigs conducted by the Bureau of Animal Industry are in close quantitative agreement. As for the other effects of inbreeding, fixation of characters and increased prepotency, these are of course in direct proportion to the percentage of homozygosis. Thus, if we can calculate the percentage of homozygosis which would follow on the average from a given system of mating, we can at once form the most natural coefficient of inbreeding. The writer[3] has recently pointed out a method of calculating this percentage of homozygosis which is applicable to the irregular systems of mating found in actual pedigrees as well as to regular systems. This method, it may be said, gives results widely different from Pearl's coefficient, in many cases even as regards the relative degree of inbreeding of two animals.

Taking the typical case in which there are an equal number of dominant and recessive genes (A and a) in the population, the random-bred stock will be composed of 25 per cent. AA, 50 per cent. Aa and 25 per cent. aa. Close inbreeding will tend to convert the proportions to 50 per cent. AA, 50 per cent. aa, a change from 50 per cent. homozygosis to 100 per cent. homozygosis. For a natural coefficient of inbreeding, we want a scale which

[3] *Genetics*, 1921, 6: 111–178.

runs from 0 to 1, while the percentage of homozygosis is running from 50 per cent. to 100 per cent. The formula $2h-1$, where h is the proportion of complete homozygosis, gives the required value. This can also be written $1-2p$ where p is the proportion of heterozygosis. In the above-mentioned paper it was shown that the coefficient of correlation between uniting egg and sperm is expressed by this same formula, $f=1-2p$. We can thus obtain the coefficient of inbreeding f_b for a given individual B, by the use of the methods there outlined.

The symbol r_{bc}, for the coefficient of the correlation between B and C, may be used as a coefficient of relationship. It has the value 0 in the case of two random individuals, .50 for brothers in a random stock and approaches 1.00 for individuals belonging to a closely inbred subline of the general population.

In the general case in which dominants and recessives are not equally numerous, the composition of the random-bred stock is of the form $x^2\ AA$, $2xy\ Aa$, $y^2\ aa$. The percentage of homozygosis is here greater than 50 per cent. The rate of increase, however, under a given system of mating, is always exactly proportional to that in the case of equality. The coefficient is thus of general application.

If an individual is inbred, his sire and dam are connected in the pedigree by lines of descent from a common ancestor or ancestors. The coefficient of inbreeding is obtained by a summation of coefficients for every line by which the parents are connected, each line tracing back from the sire to a common ancestor and thence forward to the dam, and passing through no individual more than once. The same ancestor may of course be involved in more than one line.

The path coefficient, for the path, sire (S) to offspring (O), is given by the formula $p_{o.s}=\frac{1}{2}\sqrt{(1+f_s)/(1+f_o)}$, where f_s and f_o are the coefficients of inbreeding for sire

and offspring, respectively. The coefficient for the path, dam to offspring, is similar.

In the case of sire's sire (G) and individual, we have $p_{o.g} = p_{o.s}\, p_{s.g} = \frac{1}{4}\sqrt{(1+f_g)/(1+f_o)}$, and for any ancestor ($A$) we have for the coefficient pertaining to a given line of descent $p_{o.a} = (\frac{1}{2})^n\sqrt{(1+f_a)/(1+f_o)}$, where n is the number of generations between them in this line.

The correlation between two individuals (r_{bc}) is obtained by a summation of the coefficients for all connecting paths.

Thus

$$r_{bc} = \Sigma p_{ba} p_{ca}$$
$$= \Sigma \left(\frac{1}{2}\right)^{n+n'} \frac{1+b_a}{\sqrt{(1+b_b)(1+b_c)}},$$

where n and n' are the number of generations in the paths from A to B and from A to C, respectively.

The formula for the correlation between uniting gametes, which is also the required coefficient of inbreeding, is

$$f_o = \tfrac{1}{2} r_{sd}\sqrt{(1+f_s)(1+f_d)},$$

where r_{sd} is the correlation between sire and dam and f_s and f_d are coefficients of inbreeding of sire and dam. Substituting the value of r_{sd} we obtain

$$f_o = \Sigma\, (\tfrac{1}{2})^{n+n'+1}(1+f_a).$$

If the ancestor (A) is not inbred, the component for the given path is simply $(\frac{1}{2})^{n+n'+1}$ where n and n' are the number of generations from sire and dam respectively to the ancestor in question. If the common ancestor is inbred himself, his coefficient of inbreeding (f_a) must be worked out from his pedigree.

This formula gives the departure from the amount of homozygosis under random mating toward complete homozygosis. The percentage of homozygosis (assuming 50 per cent. under random mating) is $\frac{1}{2}(1+f_o) \times 100$.

Roan Gauntlet 45276 (35284)	Royal Duke of Gloster 20901 (29864)	Grand Duke of Gloster 19900 (26288)	Champion of England (17526)	Lancaster Comet (11663)
				Virtue
			Duchess of Gloster 9th	Lord Raglan (13244)
				Duchess of Gloster 6th
		Mimulus	Champion of England (17526)	Lancaster Comet (11663)
				Virtue
			Mistletoe	Lord Raglan (13244)
				Maidstone
	Princess Royal	Champion of England (17526)	Lancaster Comet (11663)	The Queens Roan (7389)
				———
			Virtue	Plantagenet (11906)
				Verdant
		Carmine	The Czar (20947)	Lord Raglan (13244)
				Corianda
			Cressida	John Bull (11618)
				Clipper

- Comet (155)
  - Favorite (252)
    - Bolingbroke (86)
      - Foljambe (263)
        - R. Barker's bull (52)
        - Haughton
      - Young Strawberry
        - Dalton Duke (188)
        - Favorite (cow)
    - Phœnix
      - Foljambe (263)
        - R. Barker's bull (52)
        - Haughton
      - Favorite (cow)
        - Alcock's bull (19)
        - ———
  - Young Phœnix
    - Favorite (252)
      - Bolingbroke (86)
        - Foljambe (263)
        - Young Strawberry
      - Phœnix
        - Foljambe (263)
        - Favorite (cow)
    - Phœnix
      - Foljambe (263)
        - R. Barker's bull (52)
        - Haughton
      - Favorite (cow)
        - Alcock's bull (19)
        - ———

By this means the inbreeding in an actual pedigree, however irregular the system of mating, can be compared accurately with that under any regular system of mating.

As an illustration, take the pedigree of Roan Gauntlet, a famous Shorthorn sire, bred by Amos Cruickshank. This bull traces back in every line to a mating of Champion of England with a daughter or granddaughter of Lord Raglan. For the present purpose we will assume that these bulls were not at all inbred themselves and not related to each other. Since the sire traces twice to Champion of England and twice to Lord Raglan and the dam once to each bull, there are in all four lines by which the sire and dam are connected.

Individual	Common Ancestors of Sire and Dam	f_a	n	n'	$(\frac{1}{2})^{n+n'+1} \times (1+b_a)$
Roan Gauntlet 45,276 (35,284)	Champion of England (17,526)............	0	2	1	.062500
			2		.062500
	Lord Raglan (13,244)..	0	3	3	.007812
			3		.007813
					.140625

The coefficient of inbreeding comes out 14.1 per cent., a rather low figure when compared to such systems as brother-sister mating (one generation 25 per cent., two generations 37.5 per cent., three generations 50 per cent., ten generations 88.6 per cent.) or parent-offspring mating, (one generation 25 per cent., two generations 37.5 per cent., three generations 43.8 per cent., approaching 50 per cent. as a limit).

As an example of closer inbreeding, take the pedigree of Charles Collings' bull, Comet. The sire was the bull Favorite and the dam was from a mating of Favorite with his own dam. As Favorite was himself inbred to some extent, it is necessary to calculate first his own coefficient of inbreeding.

Individual	Common Ancestors of Sire and Dam	f_a	n	n'	$(\frac{1}{2})^{n+n'+1} \times (1+f_a)$
Favorite (252)	Foljambe (263)	0	1	1	.1250
	Favorite (cow)	0	2	1	.0625
					.1875
Comet (115)	Favorite (252)	.1875	0	1	.2969
	Phœnix	0	1	1	.1250
	Foljambe	0	2	2	.0312
	Favorite (cow)	0	3	2	.0156
					.4687

In the case of Comet, Foljambe and Favorite (cow) each appears twice in the pedigree of the sire and three times in the pedigree of the dam. However, only those pedigree paths which connect sire and dam and which do not pass through the same animal twice are counted. The listing of Favorite (252) and Phœnix as common ancestors eliminates all but one path in each case as regards Foljambe and Favorite cow. The remaining paths are those due to the common descent of Bolingbroke, the sire's sire and Phœnix as the dam's dam from the above two animals.

By tracing the pedigrees back to the beginning of the herd book, the coefficients of inbreeding are slightly increased. This meant going back to the seventh generation for one common ancestor of the sire and dam of Favorite. The coefficient in the case of Favorite becomes .192 instead of .188 and that of Comet .471 instead of .469. Remote common ancestors in general have little effect on the coefficient. It will be noticed that Comet has a degree of inbreeding almost equal to three generations of brother-sister mating or an indefinite amount of sire-daughter mating where the sire is not himself inbred.

THE ASSORTMENT OF CHROMOSOMES IN TRIPLOID DATURAS

JOHN BELLING AND ALBERT F. BLAKESLEE

STATION FOR EXPERIMENTAL EVOLUTION, COLD SPRING HARBOR, LONG ISLAND, NEW YORK

The present article is the one of a number of proposed papers which will deal with the behavior of the chromosomes in the different classes of Datura mutants, the correlation of the chromosomal differences with changes in structural and other characters, and with the ratios in which Mendelian allelomorphs are found in the offspring. The method mainly used in the microscopical examination, and the general principles involved, are given in two papers already in press for THE AMERICAN NATURALIST.

Sizes of Chromosomes.—The diploid *Datura Stra-*

FIG. 1. Second metaphases of a normal Datura in a pollen-mother-cell. The chromosomes are about to divide into two, and each half is constricted. (This figure, as well as Figs. 2 to 6, is a camera drawing of a preparation in iron-aceto-carmine, the cytoplasm having been thinned and flattened by appropriate pressure so that the chromosomes were in optical contact with the cover-glass.)

monium shows, in the metaphase of the second division in the pollen-mother-cells (Fig. 1), two groups, each consisting of 1 extra large chromosome, 4 large, 3 large medium, and 2 small medium chromosomes, 1 small and 1 extra small chromosome. Thus the somatic formula is 2(L+

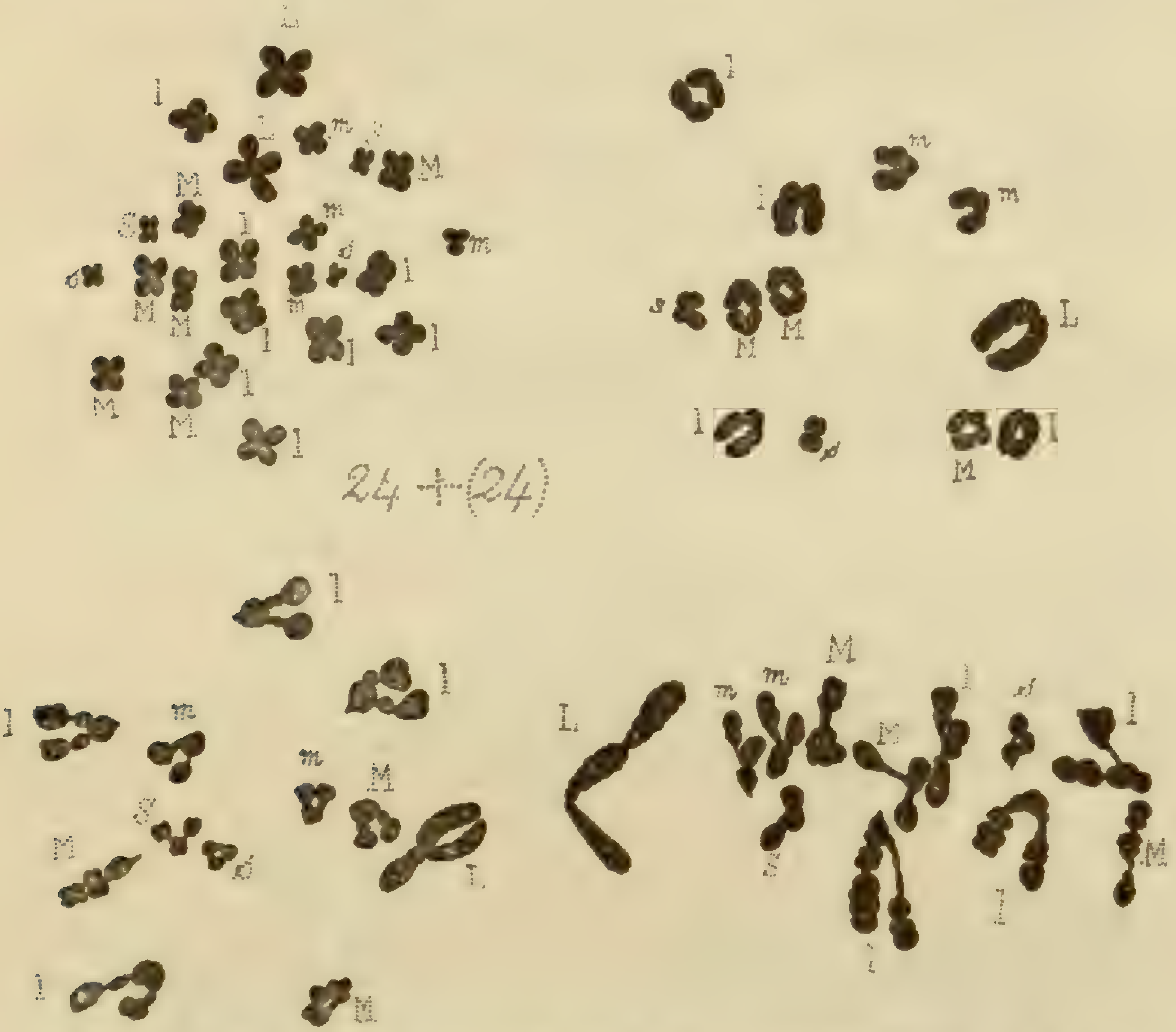

Fig. 2. One second metaphase plate of a tetraploid Datura, in a pollen-mother-cell.

Fig. 3. Late prophase of a normal Datura in a pollen-mother-cell. The size differences are especially distinct, for the smaller chromosomes have condensed earlier.

Fig. 4. Late prophase of a triploid Datura. The largest chromosome set (trivalent) was the latest to condense.

Fig. 5. Late prophase of a triploid Datura. The largest chromosome set is hook-shaped. (The late prophase or early metaphase trivalents often have the form of a ring with a handle, which is indicated in only one trivalent in Fig 4, and is not shown in Fig. 5.)

4l + 3M + 2m + S + s). Tetraploid plants have arisen, in rare cases, from these diploid Daturas (2). They show (Fig. 2) twice as many chromosomes in each of the size classes, and have the somatic formula 4(L + 4l + 3M + 2m + S + s). Out of many crosses of tetraploid

Daturas by pollen from normals, 4 triploid plants have resulted (3). Their somatic formula is shown to be 3(L + 4l + 3M + 2m + S + s). Similar results have been obtained for triploid hyacinths by de Mol (7).

Attraction of Homologous Chromosomes.—In the normal Daturas the late prophase or early metaphase of the first division in the pollen-mother-cells shows 12 sets with two united chromosomes (bivalents) in each (Fig. 3). These bivalents can readily be arranged in the six size classes. In the corresponding stage of the triploid Daturas there are 12 sets of three united chromosomes each, and these trivalents can be arranged according to the size formula (Fig. 4). Sometimes two of the three rod-shaped chromosomes are united together at both ends, and the third is joined on at one end only, or the three may form a hook (Fig. 5). Some trivalents were seen by Osawa in triploid mulberries (8), and a group of 9 trivalents was also found in a triploid Canna (1). (The complete group of 9 trivalents has also been seen in 4 other triploid Cannas.)

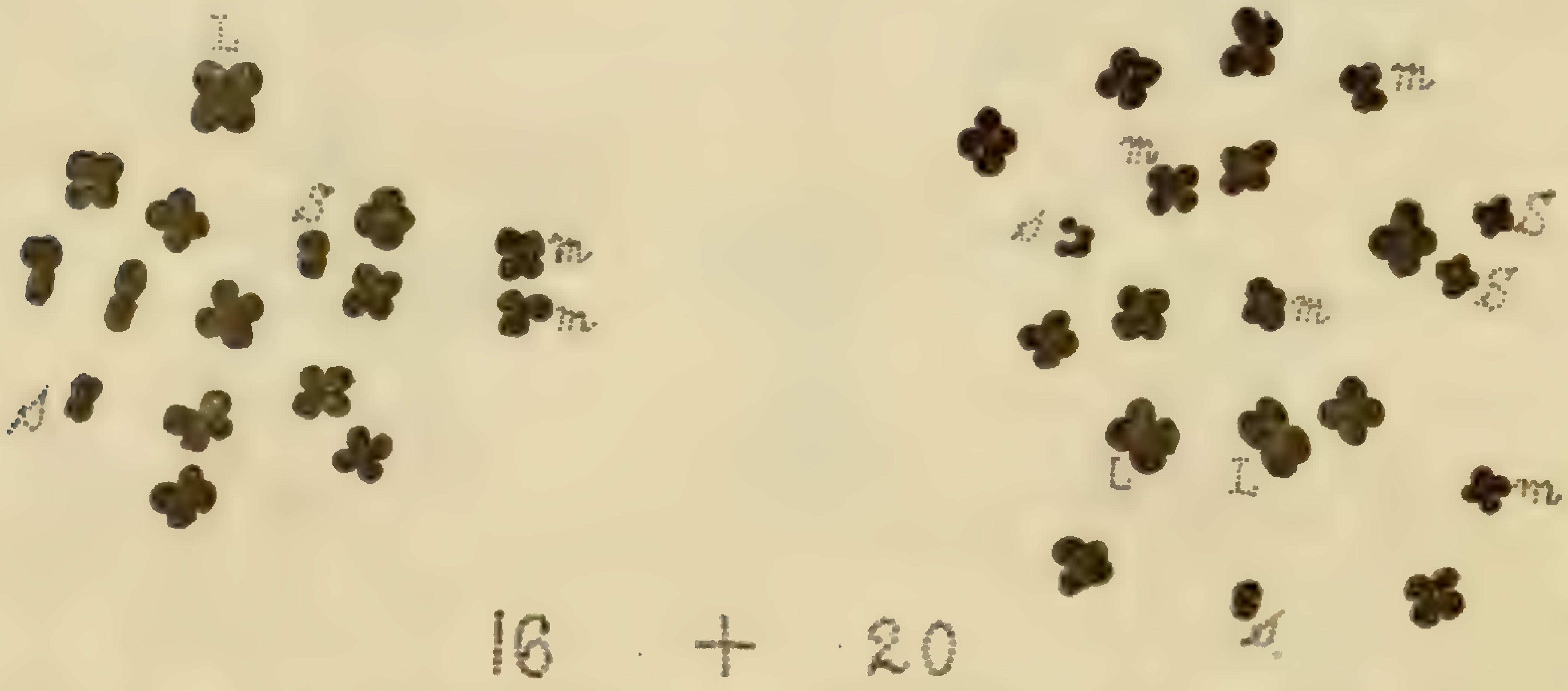

FIG. 6. Second metaphases in a pollen-mother-cell of a triploid Datura. The large and large medium chromosomes were not separable in this preparation.

Separation (Disjunction) of Chromosomes.—So far as seen in Datura, two chromosomes usually pass to one pole, and one chromosome to the other, from each trivalent, as is the case in triploid Cannas (1).

Assortment of Chromosomes.—From one triploid

plant both groups of chromosomes were counted in each of 84 pollen-mother-cells, which were in the second metaphases, and showed no detached chromosomes (Fig. 6). The assortments are given in Table I.

TABLE I

ASSORTMENT OF CHROMOSOMES IN 84 POLLEN-MOTHER CELLS OF TRIPLOID DATURA, 19729(1)

Metaphase of Second Division

Assortment of Chromosomes	12 + 24	13 + 23	14 + 22	15 + 21	16 + 20	17 + 19	18 + 18
Nos. of double groups	1	1	6	13	17	26	20
Calculated on random orientation of trivalents	0.04	0.5	2.7	9.0	20.3	32.5	19.0

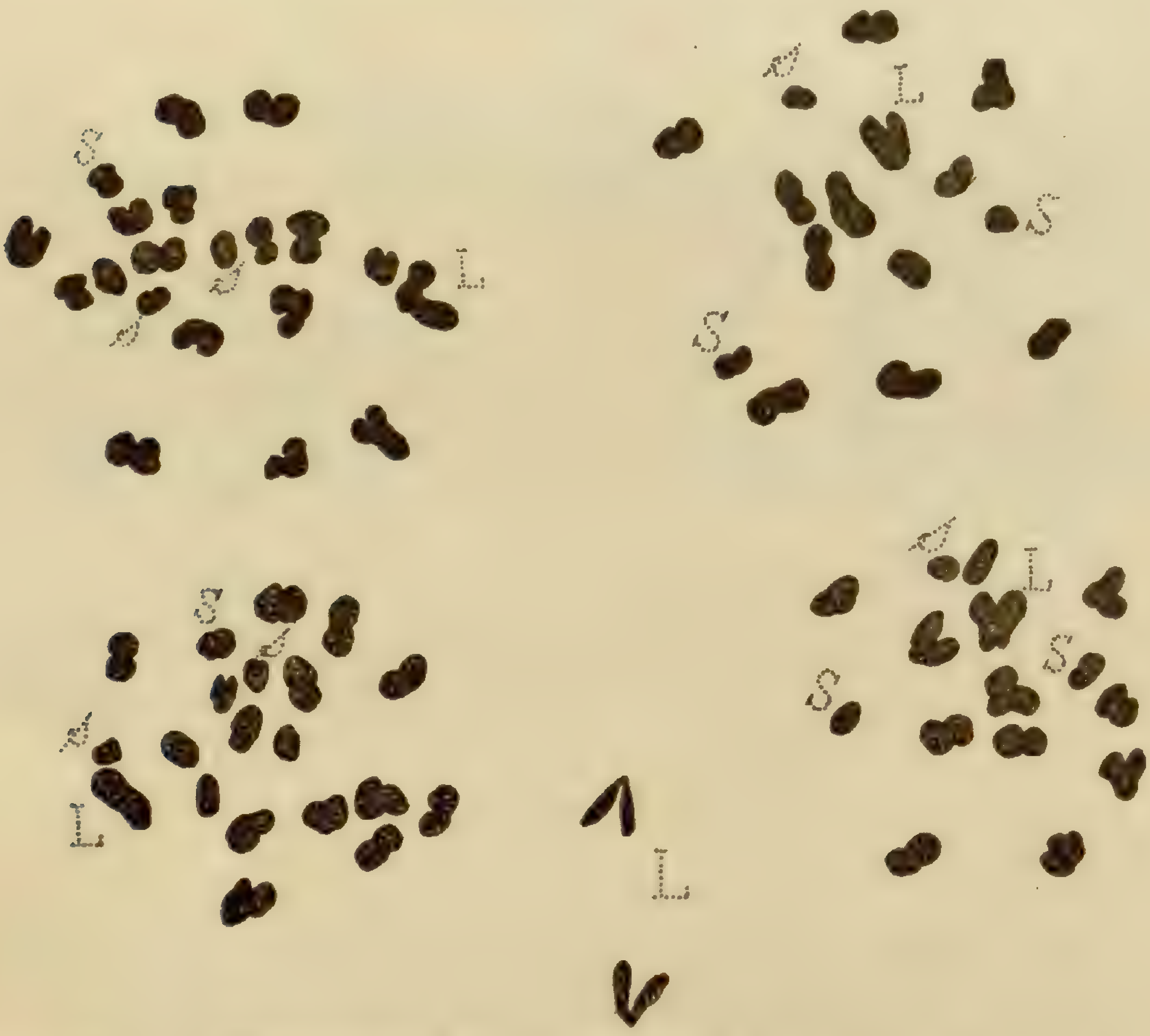

FIG. 7. Early anaphase of the second division in a pollen-mother-cell of a triploid Datura. (The upper right-hand plate was shifted upwards in drawing.) One of the 3 extra large chromosomes had apparently been detached at the first anaphase, and divided at the second division. Probably a tetrad with 2 microcytes would have resulted.

It is evident that the orientation of the trivalents in the first metaphase must be nearly or quite a random one, as was suggested in triploid Œnotheras (5, 6) and mulberries (8), and as is the case in triploid Cannas (1). (Nearly similar results were also obtained from a total of 58 single-metaphase plates from this triploid Datura.)

Detachment of Chromosomes.—Three buds yielded 62 pollen-mother-cells with both second-metaphase plates countable, and among these there were six cells showing that one chromosome had been detached at the first anaphase (Fig. 7), one cell showing detachment of two chromosomes, and one cell showing both one and two detached chromosomes. Thus there were about 13 per cent. of cases of detachment. These detached chromosomes (8) form microcytes when the pollen-mother-cells constrict to form tetrads (Fig. 8). Table II shows the numbers of microcytes seen in nearly 3,500 tetrads from 3 triploid plants. The average is 13 per cent. of cases of detachment, but the variation in different buds appears too great to be due to chance alone. In 100 pollen-grains there would be about 5 microcytes.

FIG. 8. Tetrads, etc., of a triploid Datura. Above: (1) a normal tetrad; (2) a tetrad with one microcyte; (3) a tetrad with 2 microcytes. Below: (1) a tetrad with 4 microcytes; (2) two giant cells; (3) rare form with 6 not very unequal cells.

Non-reduction.—In belated pollen-mother-cells the chromosomes in the trivalents assume the four-lobed condition of those in the adjoining cells which are in the metaphase of the second division. The first nuclear divi-

TABLE II

DETACHMENT OF CHROMOSOMES. NON-REDUCTION

Pollen Tetrads of Triploid Plants. (Percentages)

	Regular Microspores					Double-sized Microspores					
Nos. of Microspores......	4	4	4	4	4	2	2	2	Etc.	Percentage of Cases of Detachment	Nos. of Tetrads
Nos. of Microcytes.......	—	1	2	3	4	—	1	2			
Plant and Bud											
19729 (1) *a*..	67.0	20.0	9.0	0.7	0.5	2.0	0.5	0.2		30.9	403
19729 (1) *b*..	91.5	3.0	5.3	0.2						8.5	436
19729 (1) *c*..	90.3	3.3	5.9	0.2	0.2					9.6	425
19729 (1) *d*..	96.1	1.6	0.9	—	—	1.4				2.5	433
20345 (1) *a*..	83.8	7.9	8.1	0.2						16.2	444
20345 (1) *b*..	97.8	0.5	0.7	—	—	0.7			0.2	1.2	412
20345 (1) *c*..	98.0	0.8	0.3	—	—	1.0				1.1	400
20380 (1) –..	65.5	19.0	14.6	—	—	0.6	—	0.4		34.0	542
Average.....	86.3	7.0	5.6	0.2	0.1	0.7	0.1	0.1	0.03	13.0	Total 3,495
Microcytes to 100 pollen-grains = 4.9						Percentage of double-sized pollen-grains = 0.4					

sion is entirely omitted, there is no reduction (8), and two nuclei with 36 chromosomes each are formed at the second division. The two cells which result are twice the size of the average microspores, and can be seen in the pollen as giant grains. Non-reduction may be greatly increased by transient cold. It averaged 0.4 per cent. in the tetrads. A hundred full pollen-grains were measured at random from each of 8 flowers on 4 triploid plants. The average was 0.5 per cent. of giant grains.

Chromosomes of Functional Egg-cells.—In one triploid Datura, from three (or fewer) capsules pollinated by a normal, there were produced 75 mature plants, 67 of which had their chromosomes counted.

TABLE III

CHROMOSOMES OF PROGENY OF TRIPLOID DATURA POLLINATED BY DIPLOID

Nos. and Assortment of Chromosomes	12 + 12	13 + 12	14 + 12 or 13 + 13	....	24 + 12 .. 18 + 18
Nos. of plants	24	33	10	....	....
Calculated on random assortment for 4096 ovules	1	12	66	etc.	....

The number of normal progeny shown in Table III is much beyond expectation (on the hypothesis that orientation of trivalents in the first division of the megaspore mother-cell is random), even if we allow the excessive total of over 4,000 ovules to 3 capsules. Detachment of chromosomes in the megaspore-mother-cells to the maximum extent found in the pollen-mother-cells will only partially account for this excess. Similar results were obtained by van Overeem with triploid *Œnothera biennis* pollinated by the normal (9).

Triploid Inheritance.—The 75 progeny showed triploid or trisomic (not disomic) inheritance (2) of two probably independent pairs of genes, those for purple and white flowers, and those for prickly and smooth capsules.

Distribution of Extra Chromosomes.—Among the 33 plants with one extra chromosome, cases were found where this extra chromosome was extra large, large, medium, small, or extra small. These plants showed 11 bivalents and 1 trivalent at the late prophase and early first metaphase. Ten different forms were recognized by external features among 30 of the 33 forms with an extra chromosome. (Three plants have not yet been identified.) Among these ten forms, 1 form (Globe) occurred 5 times, 3 forms (Buckling, Ilex, and Reduced) occurred 4 times, 2 forms (Glossy and Elongate) occurred 3 times, 3 forms (Rolled, Cocklebur, and Poinsettia) occurred twice, and 1 form (Microcarpic) occurred once. The expectations for each of 12 possible forms are presumably equal, namely 2.5. The Datura plants with 2 extra

chromosomes so far examined showed 10 bivalents and 2 trivalents at the first prophase.

Thus the random assortment of chromosomes in triploid Daturas parallels the conclusions as to the random assortment of genes in triploid (trisomic) inheritance, and adds to the evidence for the chromosomal theory of heredity given by the cytological and genetic work on Drosophila (4) and other insects.

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ŒSTRUS AND FECUNDITY IN THE GUINEA PIG

DONALD B. TRESIDDER

Department of Anatomy, Stanford Medical School

This study was undertaken at the suggestion of Professor Meyer, primarily for determining the numerical relation between the corpora lutea of pregnancy and implantations in the guinea pig.

Most of the animals used in this experiment were purchased from dealers, for it was impossible, in the short time at my disposal, to obtain young animals of uniform age and with the exception of a few guinea pigs raised in our laboratory, only approximate ages were known.

The guinea pigs were housed in a well-lighted, sunny, heated room. Lantz, '13, reported that the optimum temperature for the guinea pig is 65°. Draper, '20, stated that they thrive best at temperatures between 50° and 70° and found young animals extremely susceptible to small changes in temperature; some of them dying when the temperature was lowered permanently from 60° to 58° F. However, I did not notice any marked difference in the behavior or condition of extremely young animals kept at a temperature of 50°. They showed every sign of vigor and no animals were lost as a result of this exposure. Indeed, I learned of guinea pigs kept in the open in unheated pens, sheltered only from wind and rain. These animals were said to thrive and to multiply at the customary rate, but no records were kept. In my own work I found that a few degrees above or below 50° seemed to make no appreciable difference in the behavior of the animals, and I hence am somewhat sceptical about the marked susceptibility of the guinea pig to cold, so often reported.

The animals were fed dry alfalfa and barley daily and green vegetables about twice a week. Many writers have reported that guinea pigs did not do well on dry feed, but it was my experience that, if fed an abundance of water, they throve on alfalfa and barley alone. Since they are subject to intestinal disturbances, it is of con-

siderable importance that they be fed with the greatest regularity.

Several animals were lost during the course of the experiments and in each case a necropsy was performed. Illness, in several of the animals, extended over a period of weeks. They lost steadily in weight, and tended to assume a characteristically crouching attitude. The fur became rough and tousled. Some of them chewed incessantly, although some pain seemed to be associated with the process. The full significance of this behavior was made clear at the necropsy. Guinea pig No. 7, for example, which succumbed after an illness of three weeks, had an empty stomach, and the abdominal cavity was absolutely devoid of fat. There were no macroscopic signs of infection or disease. Examination of the teeth revealed that the upper incisors were worn down almost to the gums, with a more than corresponding increase in the length of the lower incisors, making occlusion of the molars impossible. The molars were loose and could easily be picked from the jaw with an ordinary laboratory forceps.

The body of guinea pig No. 12, which died with practically the same symptoms, showed extreme atrophy and emaciation. Ascaroid parasites were found in the rectum. The upper incisors were loose and worn and the short stumps remaining could be be removed with the fingers. The upper and lower incisors were separated by about 8 mm., due to the fact that the molars occluded first and prevented the short, probably fractured incisors from meeting. From the findings in these cases it would seem that guinea pigs may die of starvation because of the presence of worn or irregular teeth and consequent inability to masticate food. It may perhaps be that the changes in the teeth of these animals were due to senility, but further observations are necessary to confirm this before a definite answer can be given to the question.

In order to study daily stages in the pregnancy of guinea pigs it became necessary to mate a large number of animals and to know the exact time of copulation. Stockard and Papanicolaou, '17, studied the œstrous rhythm of

the guinea pig by making microscopic examination of the material found in the vagina. They found that "Guinea pigs kept in a state of domestication and under steady environmental conditions possess a regular diœstrous cycle, repeating itself in non-pregnant females about every sixteen days throughout the entire year, with probably small and insignificant variations during the different seasons. Each period of sexual activity lasts about 24 hours and is characterized by the presence of a definite vaginal fluid which is not sufficiently abundant to be readily detected on the vulva, but is easily observed by an examination of the interior of the vagina." They added that macroscopic signs of heat are unreliable.

In my work it was found impractical to determine the existence of heat microscopically and the knowledge that heat should recur about every fifteen days furnished a starting point. Each female was given a number and entered on an individual record sheet giving the following data:

Date and hour of attempted mating.
Result of attempted mating.
Each time the animal came into heat the record showed:
Whether heat was recognizable by macroscopic examination.
Number of days since last heat.
Number of hours since the first successful coitus.
Number of hours that external signs of heat could be observed by examination.

Matings were attempted daily, whether the animal was supposed to be pregnant or not. The males were introduced into the pens with the females regardless of whether or not the latter were thought to be in heat, and they were allowed to remain with the females from five to fifteen minutes. It was easy to follow the diœstrous cycle of any individual animal. A glance at the guinea pig's record each day showed the number of days since the last heat, and, knowing that heat should return about the fifteenth day, it was practically impossible for it to come and go unnoticed unless it recurred altogether irregularly. We

found that after some practise heat could be determined rather accurately by inspection. A guinea pig in rut will often assume the position of copulation when stroked gently over the lumbar region. The vulva are swollen and moist, and often a cheesy secretion is seen. The latter is a positive sign of heat, but we found that some guinea pigs refused to mate during the entire period in which the secretion was present.

In young animals we found heat recurring every fifteen or sixteen days with very little variation among individuals of the same age. Three striking exceptions in which heat returned in twelve days will be reported later in this paper. Papanicolaou and Stockard found that in old multiparæ the period may be lengthened to 18 days. I also found that as the animals grow older they seem to become more and more irregular in their rhythm. In three very old animals I was unable to find any signs of heat throughout an entire year, although I attempted to mate them twice daily. Three other animals maintained a cycle of 20 days, and in some cases we were unable to demonstrate any regular œstrous rhythm at all, either by inspection or by the use of a male.

Subsequently (1920) these workers have reported that "underfeeding with a diet of 20 grams of carrots per day produces prolongation of the diœstrum, and at the same time a congestion in the ovary and uterus and a degeneration of developing Graafian follicles." They concluded that "the extent of prolongation of the diœstrum depends upon the stage at which an animal is underfed. . . . Large follicles seem to require better nutrition than a small primary follicle. . . . Thus a late underfeeding has a more injurious effect than an early one, and postponement of the next œstrus is correlated with a postponement of new ripe follicles in the ovary." Stockard and Papanicolaou believe that the ovarian follicles are extremely sensitive to environmental conditions. They believe that extreme variations in the œstrus cycle of certain animals may be accounted for, partially at least, by differences in nutrition.

In the course of these observations the intervals be-

tween attempted matings were shortened, with the idea that heat might be recurring unnoticed, but mating never occurred at other intervals. It is doubtful whether any definite rhythm is maintained by old guinea pigs, for pig No. 9, which was observed to be in heat December 27, was not in heat again until 49 days later. Animal No. 20 was in heat October 17 and heat did not return until 91 days later. In another instance heat returned after 118 days. However, since the age of these animals is not known, it is impossible to be sure that these irregularities are due to senility.

Bischoff, '44, stated that copulation in the guinea pig occurs within 3 hours after parturition. In four cases in which he prevented copulation heat returned after intervals of 40, 50, 51, and 51 days. Hensen, '76, and Rein, '83, claimed that the most favorable time for copulation is within one hour after parturition. I observed copulation in 12 animals immediately after parturition. Matings were attempted at one-hour intervals for six hours afterward. In four cases I was unable to mate the females at this time. They were found in heat again 34, 36, 81, and 120 days later. The first two animals were about six months old. The last two were very old, judging by their teeth. Two females mated 1 hour after parturition, 2 after 2 hours, 1 after 3 hours, 1 after 5 hours, and 2 after 6 hours. In three cases no pregnancy resulted and heat returned in 31, 31 and 29 days.

Many writers have reported that females refuse the male shortly after the first copulation. The inference is that some nervous mechanism automatically terminates heat soon after copulation. Instances have been reported in which the female refused the male 20 minutes after the first copulation. In observations extending over nearly a year, however, three cases were observed in which the female mated again eight hours after the first copulation. In the majority of cases the female permitted copulation three hours after the first mating. One animal mated 13 times in an interval of 8 hours. It seems that a female accepts the male at any time during the first stage of heat regardless of any previous intercourse, but apparently

she permits matings somewhat reluctantly after this. Instead of assuming the position for copulation when approached by the male she often runs around the cage and resists vigorously. Unless the male is very persistent and active copulation will not occur. One female resisted a second coitus for fifteen minutes by kicking, snapping, etc., only to stop suddenly and take the position for copulation. This behavior of the female may be due to previous mating or it may simply mean that the period of heat is subsiding. I am inclined to the latter view, because we have encountered many females among animals which had not been previously mated, who resisted the males vigorously for a time, only to yield in the end. The time during which the females permitted copulation unhesitatingly was a relatively short one, but after this phase had passed the animal might yet be mated if the male was persistent.

Stockard and Papanicolaou, '17, are of the opinion that among domesticated guinea pigs only a slight seasonal variation exists in the occurrence of heat, but in the present series of guinea pigs the fall months were the most favorable for matings, as shown by the following table:

Month	Number of Matings	Resulting Pregnancies	Percentage
September	23	21	91.3%
October	17	10	58.8
November	8	3	37.5
December	11	4	36.3
January	7	4	57.1
February	5	0	0.0
March	8	4	50.0
April	12	5	41.6
May	9	5	55.5
June	6	5	83.3

The males seemed to be partly responsible for this wide variation. During the winter months they were lethargic and indifferent. When placed in a pen with a female known to be in heat, the male often ignored her, eating unconcernedly instead. In many instances several males had to be placed with such a pig, in succession, before a mating took place. This is in marked contrast to the customary behavior, for when placed in a pen with two

females, the male will often go directly to the female in rut. Sometimes, however, he will mistakenly pursue the one that is not in heat, although repelled by sharp bites and other negations, only to wheel suddenly and mount the receptive female. The pursuit of the wrong animal may only serve to stimulate him, but in some instances it was necessary to remove her before he would turn his attentions to the one in rut. Puzzling sexual idiosyncrasies also were noted. Instances were observed, for example, in which a male would not under any circumstances mate with a certain female which was in heat, although he was persistent in the case of others. On the other hand, some females also were noticed to repulse a certain male, although accepting others.

It will be seen from Table I that 106 matings resulted in 61 pregnancies, or 57.5 per cent. Draper, '20, reported that only 40 per cent. of the animals bred by him became pregnant. Since Stockard and Papanicolaou found 95.4 per cent. out of 88 pigs pregnant, considerable variation would seem to exist. The large discrepancy between their results and ours may be due to the fact that the latter were working with uniformly young, selected animals or that the males were left to remain with the females, instead of being removed after several copulations.

In the many matings not followed by pregnancies, the next œstrous cycle was prolonged. This is shown by the accompanying chart.

Guinea-pig Number	Heat returned after
21	30 days.
32	44
22	28
27	15
50	46
52	29
8	30
9	31
39	12
37	15
3	29
5	30
8	15
16	30

As noticeable in the above chart, the lengthened diœstrous periods are nearly exact multiples of 15, the normal period, thus showing that the cycle is definitely periodic as reported by Stockard and Papanicolaou, '17*b*. Long, '15, found that the œstrous cycle was prolonged by inserting a glass rod in the vagina of the rat. He held this prolongation to be due to a stimulation of the cervix of the uterus. Although I stimulated the uterus of guinea pigs by means of a warm glass rod in three cases only, heat returned in 15, 15 and 16 days, and I regret that I was not able to extend this series of experiments in order to obtain more data on this interesting phenomenon revealed by Long in the rat. However, from the above table, it is clear that copulation definitely prolongs the next œstrous cycle in the majority of cases. This may be due to direct stimulation of the cervix of the uterus, as explained by Long, or implantation may have occurred, followed by abortion or by absorption of the young conceptuses, in cases in which the period was greatly prolonged.

Guinea pig No. 39 (see Table II) was mated two hours after parturition, but no pregnancy followed. This animal was remated 12 days later, with resulting pregnancy. This confirms a case reported by Rubasckhin, '05, in which heat returned 10 days after parturition. Stockard and Papanicolaou, in considering Rubasckhin's report, regarded 10 or 12 days as too short a period to indicate the return of heat. Nevertheless, in the case reported here heat was unmistakable, and this animal which was mated 12 days after parturition became pregnant. I observed heat to return in 12 days also in two other pigs.

Young animals constantly in association with males became pregnant at an earlier age than females isolated from males. Of a litter containing 3 females and 1 male, two females were placed in separate cages a few days after birth and the remaining male and female were allowed to run together. At the age of 5 months, the latter produced a litter. This indicates that the mating of this pair occurred before the animals were three months old. Yet no ill effects of this early mating or of the inbreeding could be detected in the offspring.

When the two sisters were two months old, males were introduced into the pens twice daily, but no signs of heat were observed, and no matings occurred until these females were five months old. Similar results were obtained with two other litters. Since my work was done Mr. Warnock, a fellow student, has observed two females to bear viable litters at the end of the third month. This implies mating at the early age of one month. The paternal male was several months older, however.

The Corpora Lutea of Pregnancy

In order to study the correlation between corpora lutea and implantations during the various stages of pregnancy, animals were mated and killed, from the seventh day of gestation on, for each day up to and including the fifteenth. From the fifteenth day to full term, animals were killed every other day.

When the guinea pigs were killed, the ovaries and uteri were removed and placed in formalin for twenty-four hours and the number of embryos in each horn of the uterus recorded. The ovary corresponding to the horn of the uterus having the larger number of conceptuses was arbitrarily chosen for use in determining what relation might exist between the number of conceptuses and the number of corpora lutea. Thus guinea pig No. 10 had two conceptuses in the right horn and one in the left. The right ovary was embedded and cut serially into thick sections. The left ovary was cut 7 micra thick for the study of changes in the corpora lutea during pregnancy.

In a study of 14 embryos, Draper, '20, found 76 in the left horn and 69 in the right, a ratio of 1 to 0.9. Of 98 embryos from 35 guinea pigs, I found 55 in the right horn and 43 in the left, a ratio of 1 to 0.78. The average number of fœtuses per pregnancy was three.

Table II shows that there is a marked agreement between the number of corpora lutea in an ovary and the number of implantations in the corresponding horn of the uterus. Out of 34 ovaries examined, the number of corpora lutea was the same as the number of embryos

in the corresponding horn of the uterus in all save six cases. In five of these six instances there was one embryo less in the horn of the uterus than there were corpora lutea in the ovary. In the other case, the right

TABLE II

Guinea Pig	Duration of Pregnancy	Embryos		Corpora Lutea		Remarks
		Right	Left	Right	Left	
35	7	1	1	3	1	Well-formed C.L. but no external evidence of implantations.
34	8	0	3	1	3	
33	9	2	1	2	1	
32	10	1	3	1	3	
31	11	1	1	1	3	Well-marked evidence of resorption.
30	12	3	0	3	0	
29	13	1	2	1	2	
28	14	3	1	3	1	
27	15	0	1	0	2	
26	17	3	0	2	0	
25	19	1	3	1	3	
24	21	2	1	2	1	
23	23	2	3	2	3	
22	25	3	0	3	0	
21a	27	3	0	2	0	
20	29	3	1	3	1	
19	31	2	1	2	1	
18	33	2	0	3	1	
17	35	2	1	2	0	
16	37	3	1	3	1	
15	39	2	1	2		
14	41	1	2	1	2	
13	43	1	2	1	2	
12	45	1	2	2	2	Conceptus on left side almost completely resorbed.
11	47	1	1	2	1	
10	49	2	1	2	1	
9	51	2	1	2	1	
8	53	1	2	1	2	
7	55	2	0	2	0	
6	57	1	1	2	1	
5	59	1	2	2	2	
4	61	2	1	2		
3	63	3	1	3		
2	Term	2	1	2	1	
A	23	0	0	2	0 A	C.L. of pregnancy but no implantations found.
B	45	0	0	1	1 B	

horn showed 3 embryos although only two corpora lutea of pregnancy were present in the ovary. Hence, in this case, two embryos developed from a single ovum or a single follicle contained two ova. In the instances where there was one more corpus luteum than embryos it is possible that another conceptus was present and became

absorbed or that an ovum degenerated before implantation, or that it failed of fertilization.

As shown by Meyer, '17 and '19, and Stockard and Papanicolaou, '18, absorption is not uncommon in the uteri in guinea pigs. In this series, three embryos which were clear-cut cases of absorption were found upon examination of the uteri after their removal. In No. 12, which was killed forty-five days after copulation, two normal embryos were found in the left horn, but in the right horn there was nothing but a small mass which had undergone almost complete absorption.

According to Stockard and Papanicolaou, '18, embryos eight or ten days old may be detected by "carefully feeling the uterus through the body wall of the mother." They report a case as follows:

> A normally developed embryo 19 mm. crown rump length is shown in Fig. 6 and near it is seen an amorphous embryonic mass 2 mm. in longest diameter which represents the other member of the litter. . . . The entire mass of the smaller ovum in the uterus was about that of a ten-day specimen, while the normal individual was a typical 20-day specimen. This case was detected by external examination and was merely opened in order to use the embryos for illustrating the phenomenon.

Although I used the method of Papanicolaou and Stockard in palpating guinea pigs, in no instance was I able to determine the number of embryos with certainty under fifteen days. Because of this fact, I found it necessary to sacrifice the animals in order to determine the number of implantations before this period.

Guinea pig No. 35 and guinea pig No. 34 were killed seven and eight days after conception, respectively, and the uteri removed. Careful palpation of the removed uteri failed to reveal the number of conceptuses. The uteri were then opened, but in order to determine the number of implantations present it was necessary to embed them and make serial sections. From this I am led to question the possibility of determining the number of embryos in the uterus by palpation through the abdominal wall on the eighth to tenth day of pregnancy. This skepticism seems warranted, further, by the measurements of

three ten-day conceptuses, *6.5 × 3 mm.*; *6.8 × 4.5 mm.*; *6.5 × 4.5 mm.* respectively. Draper gave the estimated length of an 11-day *embryo* measured under magnification as 2 mm.

Stockard and Papanicolaou (1918) likewise reported that a "slightly cystic ovary" has frequently been diagnosed by palpation through the abdominal wall of the guinea pig. In my observations 23 out of 75 ovaries were found to be cystic; but the largest cyst measured only *1.6 mm.× 1.68 mm.* and not even this could by any chance have been palpated through the abdominal wall. Hence, it would seem that Stockard and Papanicolaou must have been dealing with markedly large and unusual, rather than with slightly, cystic ovaries.

From a study of a large series of gestations in the domestic pig, Corner, '21, concluded that internal migration of ova is relatively common. This small series of pregnancies in the guinea pig furnishes very little evidence upon this question, for such a possibility is suggested only by No. 17, a pregnancy of 35 days in which there were 2 corpora and 2 implantations on the right side and no corpora but one implantation on the left side. Since the total number of implantations in this case exceeds that of corpora, one must assume that one ovum divided or that one follicle contained ova and that one of the ova arising from the right then migrated to the left cornu. However, since this pregnancy was so far advanced, this assumption implies that a corpus luteum of pregnancy in the guinea pig can not be wholly resorbed in 35 days and that it never fails to form.

It is of special interest in this connection that a second case of this kind has been observed in this laboratory by Miss Clark. In this case there were two corpora in the left ovary and none in the right, with one implantation on each side. Since this pregnancy was only 17 days old, the question of early resorption of the corpus luteum probably can be excluded with considerable certainty but that of failure of the corpus luteum to form, remains.

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VARIATIONS IN THE NUMBER OF VERTEBRÆ AND OTHER MERISTIC CHARACTERS OF FISHES CORRELATED WITH THE TEMPERATURE OF WATER DURING DEVELOPMENT

CARL L. HUBBS

Museum of Zoology, University of Michigan

I

For several years I have been studying the correlations between altered environmental conditions and the number of vertebræ and other segmentally arranged structures in fishes. Johannes Schmidt, of the Carlsberg Laboratory in Copenhagen, has been carrying on a series of intensive investigations (see bibliography) which deal with the same problem, and which are for the greater part rather closely paralleled by my own studies. Both of us have obtained, independently, a rather large volume of experimental and observational evidence indicating that the meristic characters displayed by an individual fish are determined not alone by heredity, but in part also by the environmental conditions, particularly temperature, which prevail during some sensitive developmental period.

II

The present study is one of those comprising the series just mentioned. It deals with variations in the number of vertebræ, scale-rows and fin-rays within one year-class and between two successive year-classes of the lake "shiner," *Notropis atherinoides* (Cyprinidæ), and in comparison between the corresponding year-classes of the "blue-gill" sunfish, *Lepomis incisor* (Centrarchidæ). These variations appear to be correlated with differences in temperature prevailing during the several developmental periods involved.

The material of each species is probably a unit as re-

gards "race." It was all obtained in a lagoon in Jackson Park, Chicago, during the third week of December, 1919. At this time what seemed to be the entire fish population of the lagoon was congregated in an opening, about five meters wide, in the ice along shore. These fishes showed symptoms of asphyxiation. They were so abundant that at times, while they were gyrating about, the mass of fishes below would force the almost solid upper layer a centimeter or two above the surface over an area of perhaps a square meter. A water bucket was filled with fishes, mostly *Notropis atherinoides,* by two or three sweeps of a small hand-net. More than one thou-

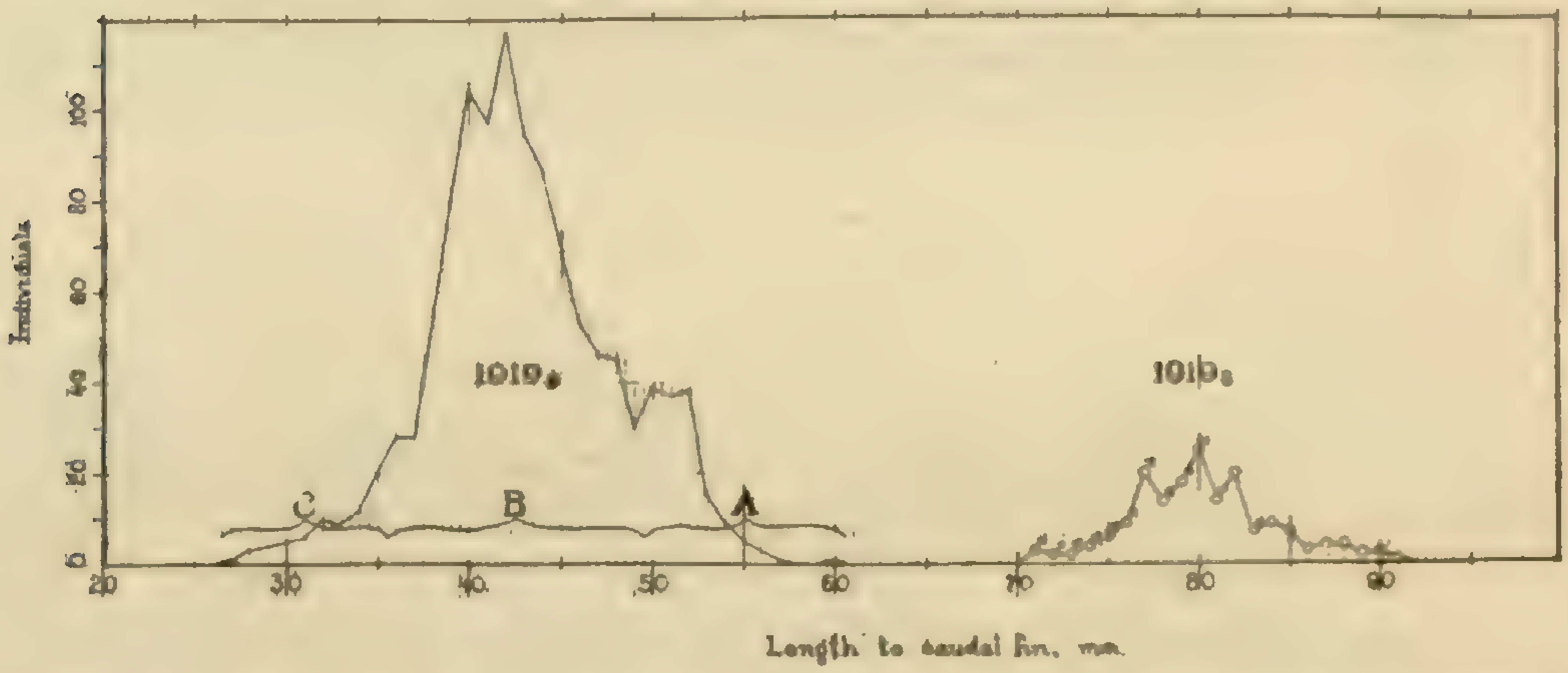

Fig. 1. Frequency graph, indicating the year-classes of *Notropis atherinoides.*

sand of the young of that year (1919) of the Notropis were saved after random selection, and preserved for study with all older fish of the same species. All of the sunfishes (*Lepomis incisor*) obtained at the same time and place were preserved and studied. Of the two species, the sunfishes belonged to a population practically confined to the lagoon, while the minnows had moved into the lagoon, late in the preceding autumn, from the more open waters of Lake Michigan.

The specimens thus obtained were grouped into year-classes. Age determinations were made by the usual methods of counting the annuli (winter lines) on the scales, and as a check the seasonal bands of the otoliths, and furthermore by the preparation of a frequency graph

from the length measurements of the entire material. The young of the year (obtained in 1919) are referred to as the 1919_9 class; those of the previous year as the 1919_8 class, and so forth. The 1919_9 year-class of the *Notropis atherinoides* is further divided into three subclasses, A, B and C, named in the direct order of hatching, hence in

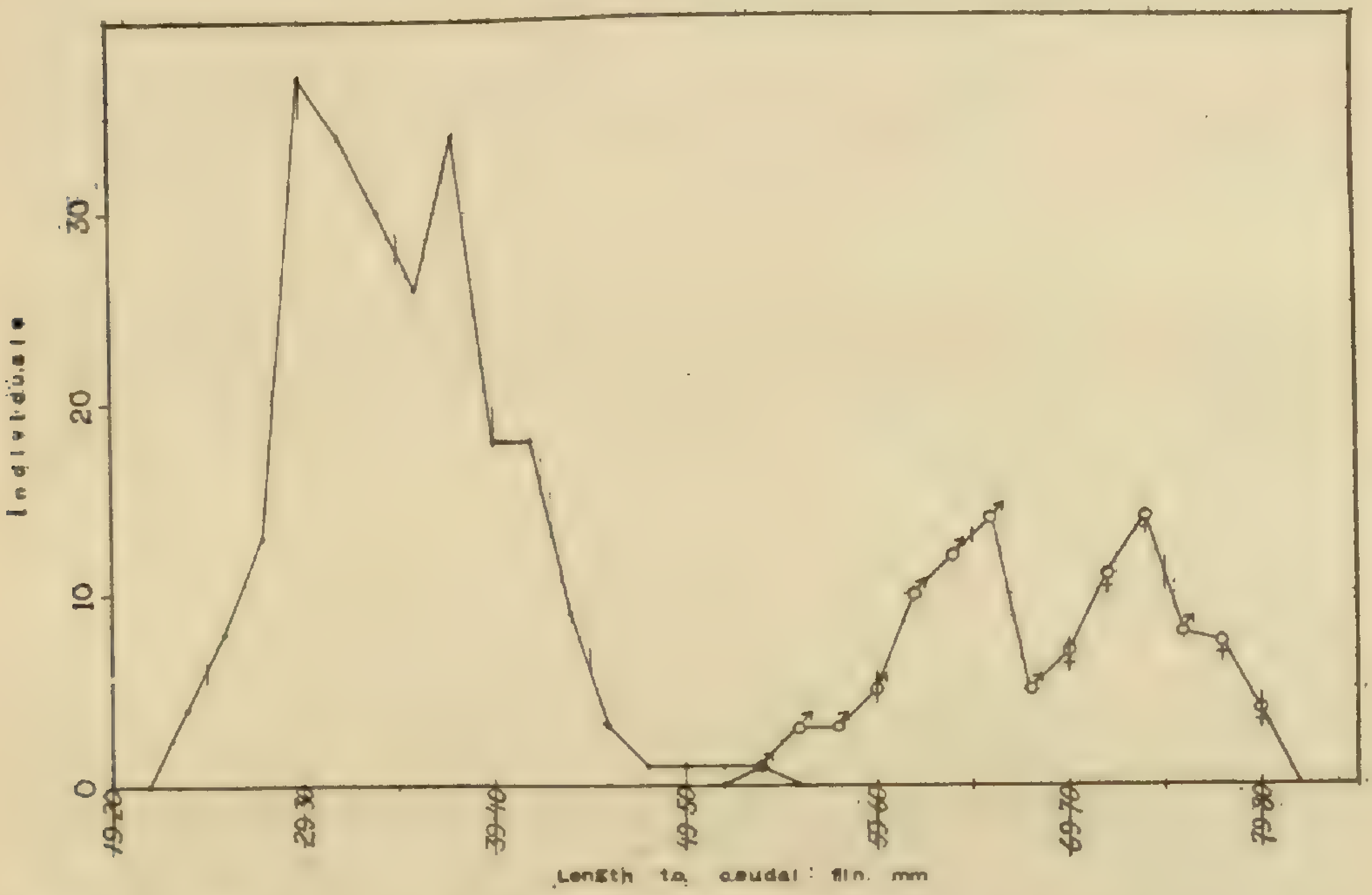

FIG. 2. Frequency graph illustrating the year-classes of *Lepomis incisor.*

the indirect order of size. The year-classes for both species are indicated on the graphs forming Figs. 1 and 2. The symbols on the curve for the 1919_8 class of each species indicate the sex predominant among the representatives of each size.

III

A series of water temperatures appear unavailable, but in the case of such a shallow, nearly enclosed lagoon the air temperatures of the region may safely be substituted. Hence the *Climatological Data* (Illinois Division, 1918 and 1919) for Chicago were used in constructing Fig. 3; the temperatures given for each week were obtained by averaging the daily means.

On the temperature chart there are indicated the periods of development for each of the two species as ob-

served at the same locality in 1919. The data for *Lepomis incisor* seem satisfactory (see Hubbs, 1919), but those for *Notropis atherinoides* are less complete and more circumstantial. In the case of the minnow, the developmental period is divided into three periods (A, B and C) corresponding with the three subclasses into which the 1919 year-class has been divided. Period A followed an inshore spawning migration of the mature individuals, coincident with the rapid rise in temperature during March; period C preceded the withdrawal of the breeding stock from the shore waters of the lake; the intervening period is termed B.

The limited field observations on the spawning and developmental period for *Notropis atherinoides* during 1919 are, fortunately, strongly confirmed by a study of

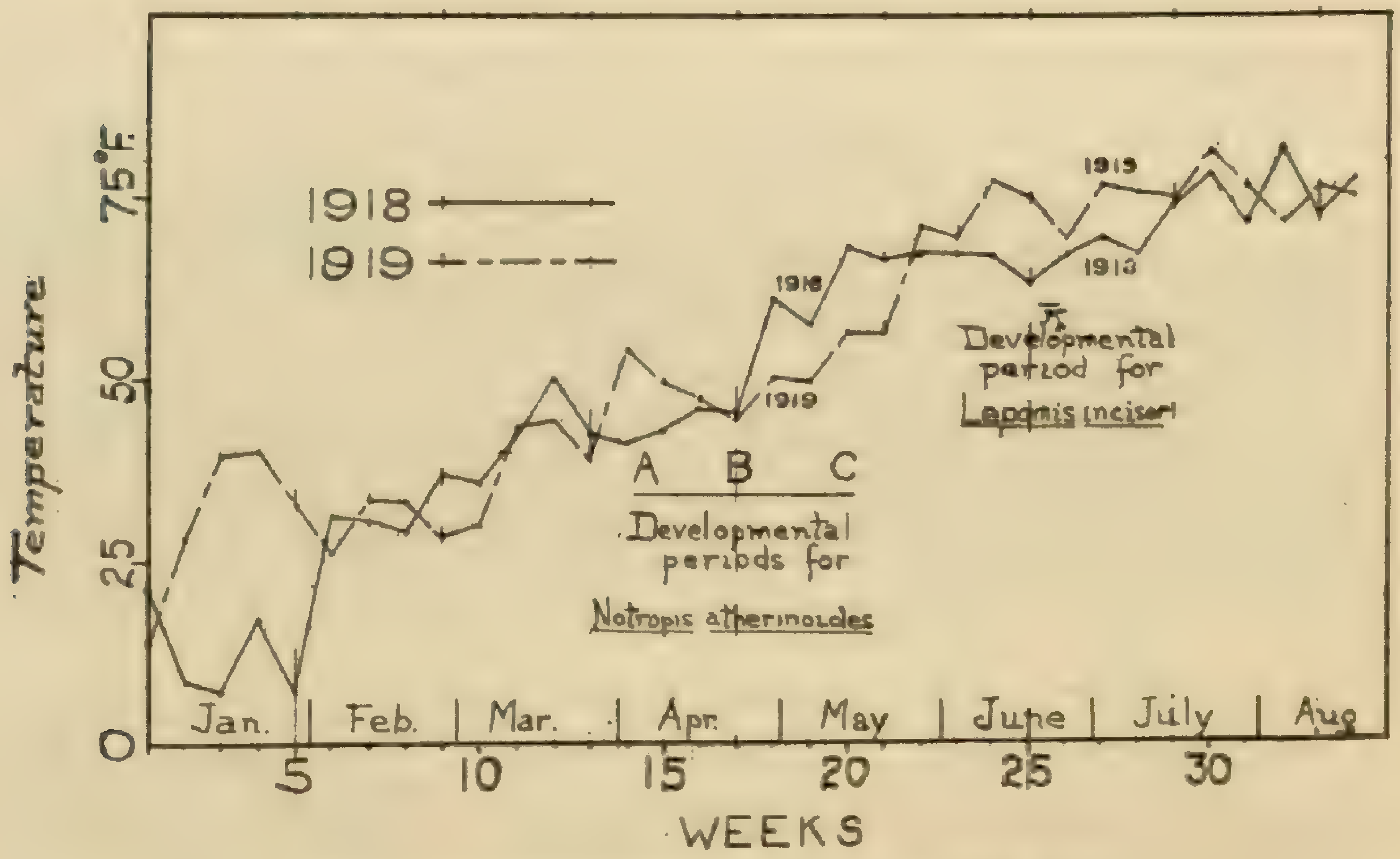

FIG. 3. Air temperature at Chicago, 1918–1919.

the scales. The scales of the largest specimens taken in December, namely those comprising subclass 1919_9A, and forming a distinct mode in the frequency graph (Fig. 1), show a well marked nuclear area of weak concentrated circuli indicative of retarded growth, followed by the coarser, more regular circuli indicative of normal summer growth. This initial period of retarded growth presum-

ably corresponds with the cold period in April (see Fig. 3). The scales of the medium-sized specimens (subclass B) show on the average a narrower nuclear area suggesting slackened growth. It is presumed that these individuals passed through their early development toward the end of this cold period. The scales of the smallest specimens, those of subclass C, show no such nuclear area of weak concentrated circuli. These fishes supposedly developed during the warm weather of May.

The data on the developmental period of these two species for the preceding breeding season (1918) are less complete than those for 1919, yet not wholly lacking. *Lepomis incisor,* at least, bred during the corresponding weeks in both years (but in less abundance in 1918 than in 1919).

A comparison of the available observational data with the temperature chart (Fig. 3) indicates that, on the average, the developmental period for *Notropis atherinoides* was colder in 1919 than in 1918, whereas these temperature relations were distinctly reversed in the case of *Lepomis incisor,* and furthermore, that the temperature was distinctly higher at the beginning and toward the close of the 1919 breeding season for the Notropis, than during the middle of this period.

IV

These differences in the developmental temperature appear to be correlated with variations in the number of segments in the case of both fishes. Comparisons will first be made between the two year groups of *Notropis atherinoides,* then between the same year groups of *Lepomis incisor,* and finally between the three subclasses into which the 1919 brood of the Notropis has been divided.

The vertebræ in the 1919_9 class of *Notropis atherinoides* are sufficiently more numerous on the average than those of the 1919_8 class to shift the modal number from 41 to 42, the average from 41.41 ($\pm$0.04)[1] to 41.74 ($\pm$0.015).

[1] The probable error of the average.

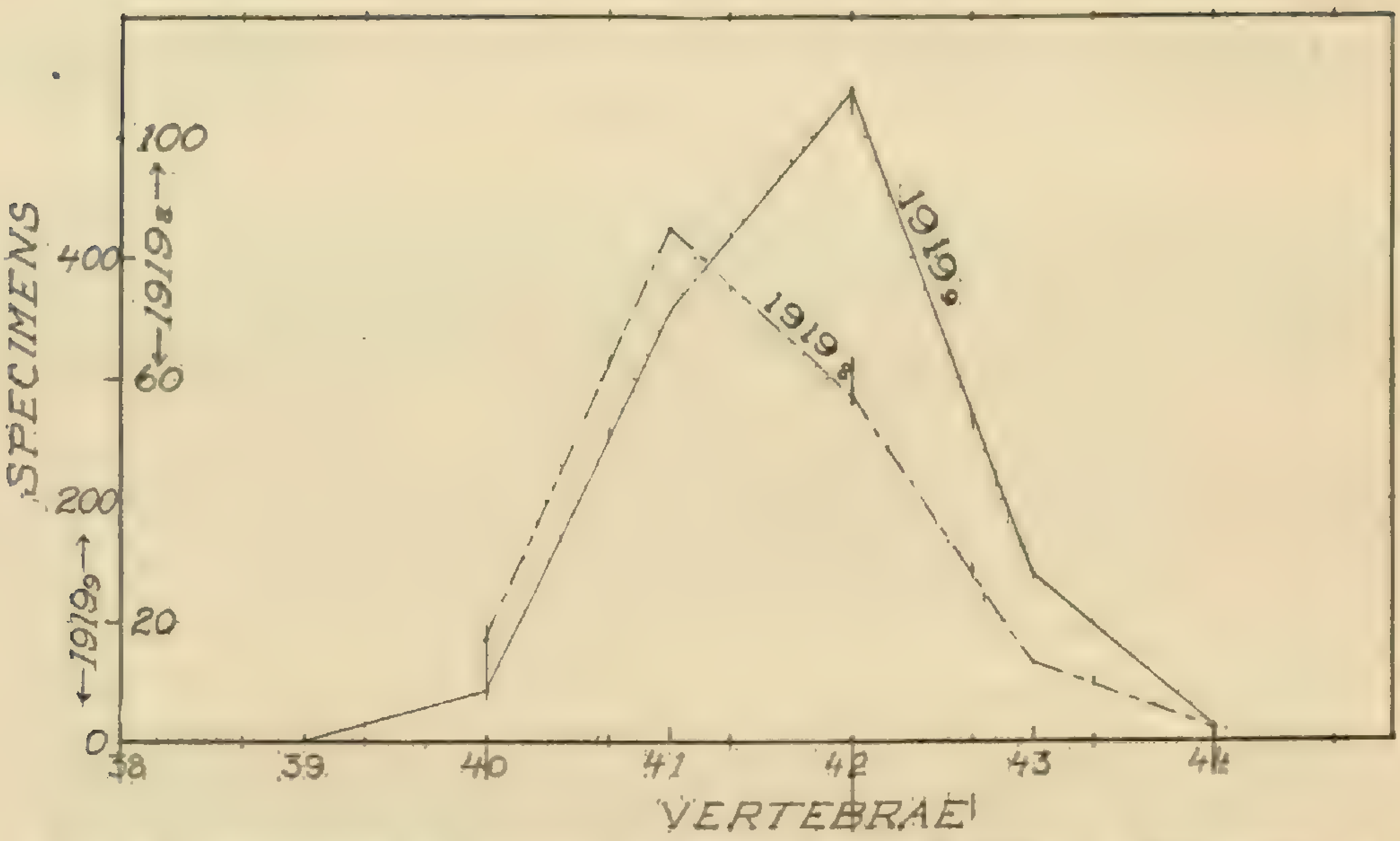

FIG. 4. Comparison of number of vertebræ in successive year-classes of *Notropis atherinoides.*

The portion of the vertebral column affected is the caudal, not the precaudal (abdominal) division: the averages for the precaudal vertebræ are 22.82 ($\pm$ 0.02) for 1919_8 and 22.85 ($\pm$ 0.01) for 1919_9 for the caudal vertebræ, 18.60 ($\pm$ 0.035) for 1919_8, and 18.87 ($\pm$ 0.01) for 1919_9. Similarly, the number of scales in the lateral line averages higher in the 1919_9 lot: the modal number is 40 rather than 39 as it is in 1919_8 class; the average number is 40.05 ($\pm$ 0.04) rather than 39.65 ($\pm$ 0.04). The modal number

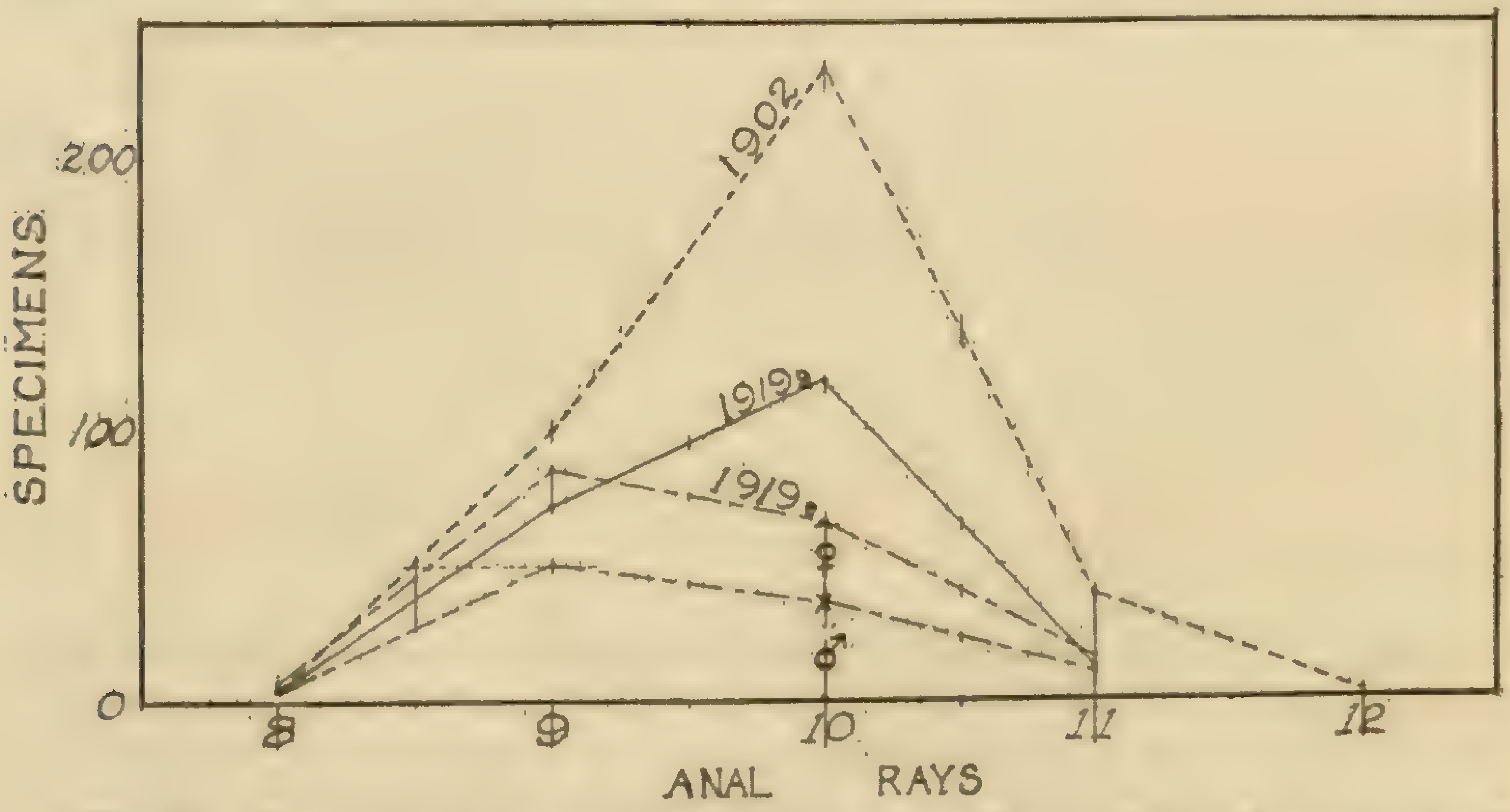

FIG. 5. Comparison of variations in number of branched and anal rays in different year-classes of *Notropis atherinoides.*

of branched anal rays[2] is 10 in the 1919_9 series, 9 in the 1919_8 class; the averages are 9.52 ($\pm$ 0.05) for 1919_8 males,

FREQUENCY TABLE I

COMPARISON OF THE MERISTIC FEATURES OF THE 1918 AND 1919 BROODS OF *Notropis atherinoides*

Character: Total Number of Vertebræ

Year-class	39	40	41	42	43	44
1919_8	—	17	85	57	13	2
1919_9	1	43	356	539	137	12

Number of Precaudal Vertebræ

Year-class	21	22	23	24	25
1919_8	3	38	121	13	—
1919_9	2	240	766	78	1

Number of Caudal Vertebræ

Year-class	17	18	19	20	21
1919_8	6	74	80	16	—
1919_9	6	269	661	142	10

Number of Scales in Lateral Line

Year-class	37	38	39	40	41	42	43	44	45
1919_8	2	26	95	92	34	11	2	—	—
1919_9	—	22	90	164	66	21	9	2	1

Number of Branched Anal Rays

Year-class	8	9	10	11	12
1902	4	100	232	39	2
1919_8 ♂	4	51	37	10	—
1919_8 ♀	3	34	29	6	—
1919_9	4	72	118	12	1

[2] The last ray as usual was counted as double, *i.e.*, as divided to the base. Occasionally the posterior half of this divided ray is again divided well toward the base. In fact a complete transition can be traced between fins having a given number of rays with those having one more ray. It is highly improbable, however, that this transition is sufficiently frequent as to permit a serious modification of the average number of rays, through a personal error in counting.

9.53 ($\pm$ 0.06) for 1919_8 females, and 9.69 ($\pm$ 0.03) for both sexes of the 1919_9 class; in material collected in 1902 in the same lagoon the average is still higher, 9.83 ($\pm$ 0.02). The data on which these figures are based is given in Frequency Table I. In all three characters, namely the number of vertebræ, of scales along the lateral line, and of branched anal rays, the year-class developed in the cooler season displays a significantly higher average.

A highly similar yet exactly reverse condition is displayed in the analysis of the counts on the *Lepomis incisor* material. In this case the total number of vertebræ, and the number of caudal, but not precaudal, vertebræ; the number of dorsal spines, dorsal soft-rays, anal soft-rays, and hence the total number of vertical fin-rays, all

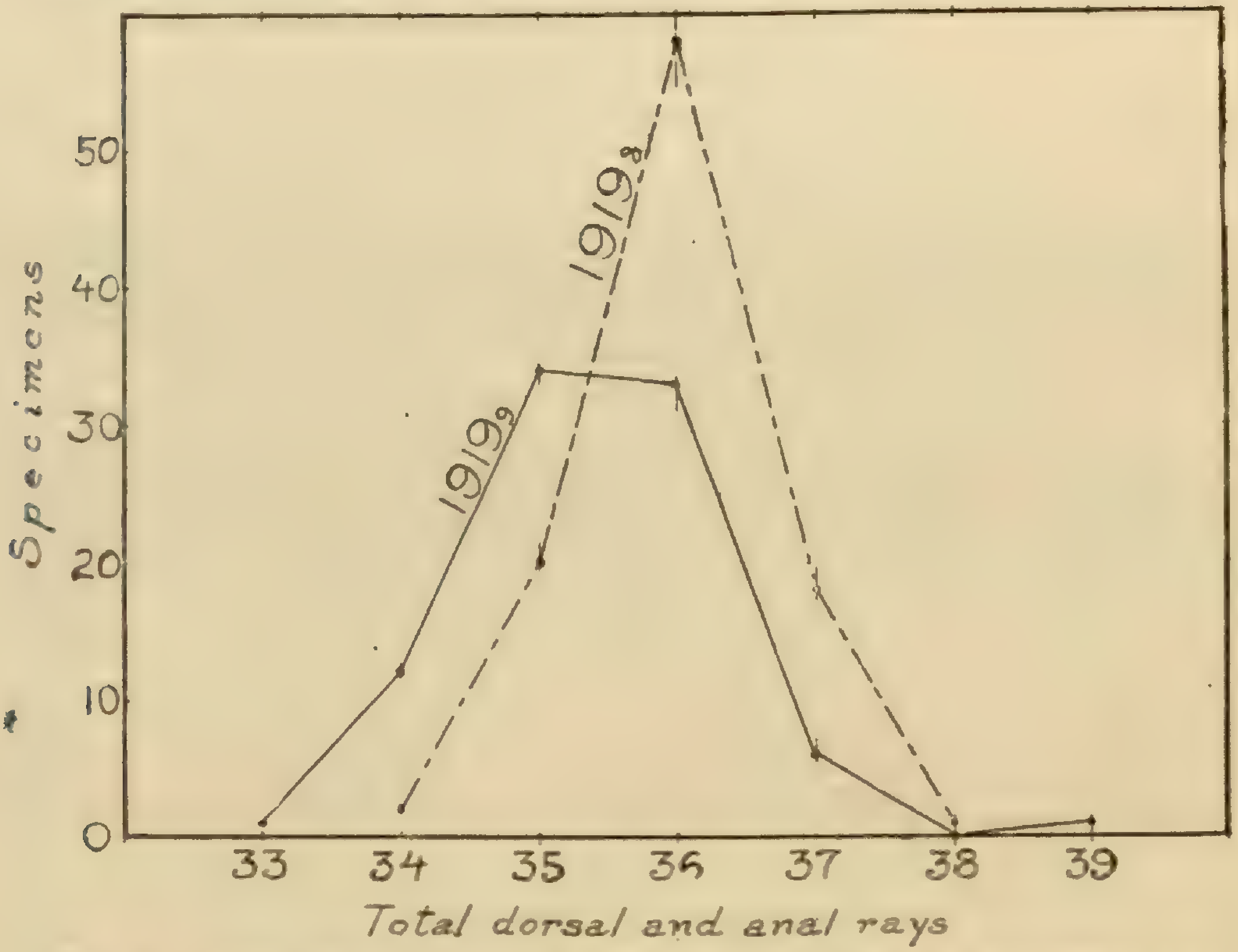

FIG. 6. Comparison of number of dorsal and anal fin rays in successive year-classes of *Lepomis incisor*.

average higher in the class born in 1918 than in that of 1919. But we noted above that the temperature relations during the developmental periods of the two years were likewise reversed. In both *Notropis atherinoides* and

FREQUENCY TABLE II

COMPARISON OF THE NUMBER OF VERTEBRÆ IN THE 1918 AND 1919 BROODS OF *Lepomis incisor*

Year-class	Character			Average	Probable Error
	Total Number of Vertebræ				
	28	29	30		
1919_8	—	95	9	29.10	0.02
1919_9	7	219	8	29.00	0.00
	Number of Precaudal Vertebræ				
	11	12	13		
1919_8	2	100	2	12.00	0.01
1919_9	2	230	2	12.00	0.00
	Number of Caudal Vertebræ				
	16	17	18		
1919_8	—	95	9	17.09	0.02
1919_9	7	219	8	17.00	0.01

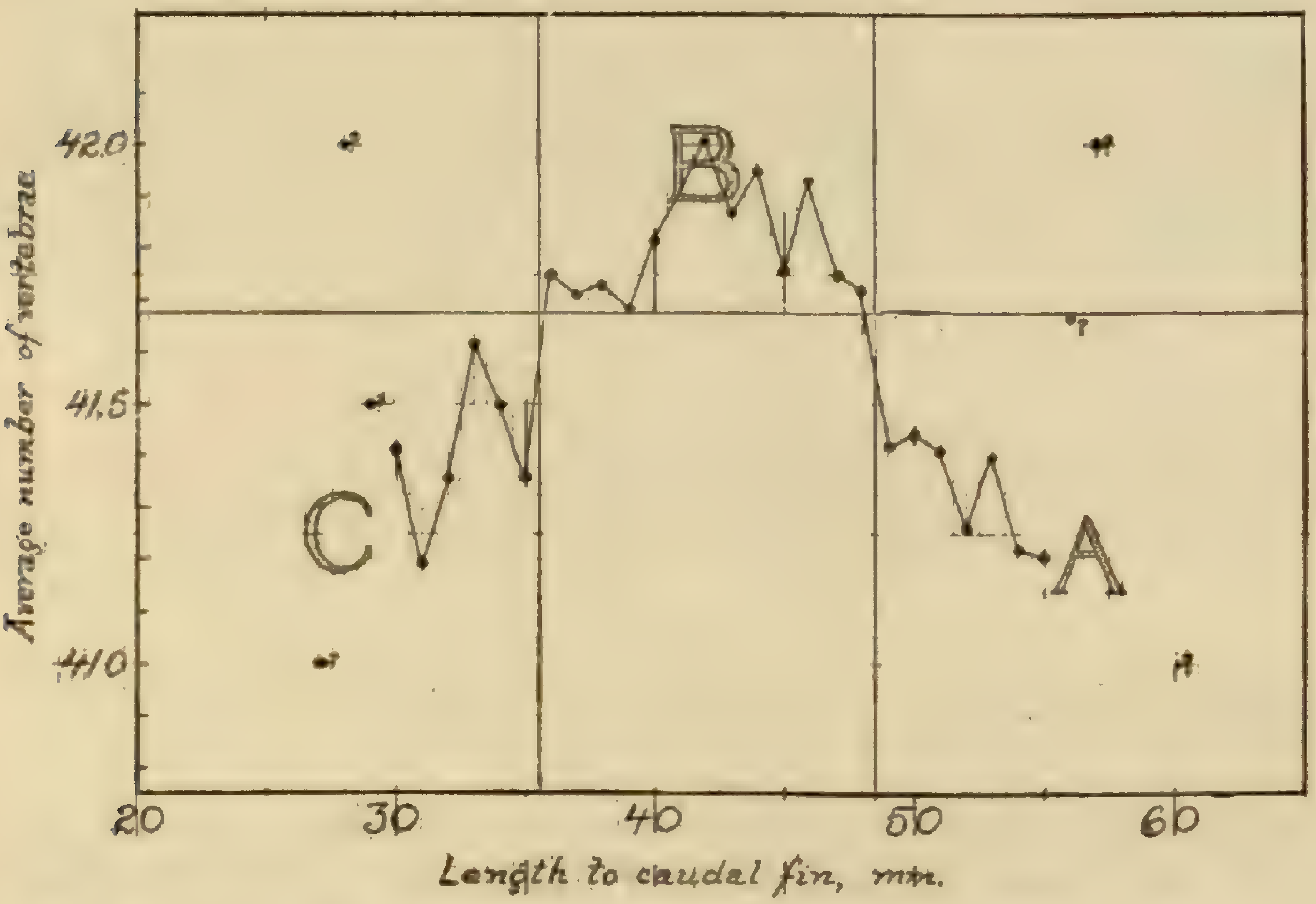

FIG. 7. Illustrating seasonal variation in number of vertebræ in *Notropis atherinoides*.

Lepomis incisor, therefore, a higher number of segments was developed in the year class developed at the lower temperature. The detailed data are given in Frequency Tables II and III.

Evidence has already been given indicating that the 1919 year-class of *Notropis atherinoides* is divisible into three subclasses, of which the middle (B) developed during colder weather than either the first (A) or the last (C). The data given in Frequency Table IV and in figure 6 demonstrate that this subclass B possesses a decidedly higher number of vertebræ than either of the other two. The averages are as follows: for the 146 specimens of subclass A, 41.38 ($\pm$0.04); for the 845 comprising subclass B, 41.84 ($\pm$0.02); for the 97 individuals of subclass C, 41.42 ($\pm$0.05).

FREQUENCY TABLE III

Comparison of the Number of Fin-rays in the 1918 and 1919 Broods of *Lepomis incisor*

Year-class	Character							Average	Probable Error
	Number of Dorsal Spines								
	IX	X	XI	XII					
1919_8	1	79	22	—				10.21	0.03
1919_9	2	74	11	1				10.125	0.03
	Number of Dorsal Soft-rays								
	10	11	12	13					
1919_8	1	37	63	—				11.61	0.03
1919_9	3	51	32	2				11.375	0.04
	Number of Anal Soft-rays								
	9	10	11	12					
1919_8	—	2	80	19				11.17	0.03
1919_9	1	12	70	5				10.90	0.03
	Total Rays in Dorsal and Anal Fins								
	33	34	35	36	37	38	39		
1919_8	—	2	20	58	18	1	—	35.96	0.05
1919_9	1	12	34	33	6	—	1	35.40	0.07

FREQUENCY TABLE IV

VARIATION IN NUMBER OF VERTEBRÆ WITHIN ONE-YEAR CLASS OF *Notropis atherinoides*

Sub-class	Size Group	Number of Vertebræ						Average	Probable Error
		39	40	41	42	43	44		
1919_9 C....	27	—	—	1	—	—	—	(41.00)	—
	28	—	—	1	1	1	—	(42.00)	0.32
	29	—	—	2	2	—	—	(41.50)	0.29
	30	—	—	9	4	—	—	41.31	0.09
	31	—	1	5	3	—	—	41.22	0.16
	32	—	2	7	4	—	1	41.36	0.175
	33	—	1	4	7	1	—	41.62	0.145
	34	—	1	7	10	—	—	41.50	0.095
	35	—	1	13	7	1	—	41.36	0.09
1919_9 B....	36	—	—	11	13	4	—	41.75	0.09
	37	—	1	16	18	6	—	41.71	0.07
	38	—	5	10	36	5	—	41.73	0.05
	39	1	4	24	39	8	1	41.68	0.06
	40	—	3	28	44	17	—	41.82	0.05
	41	—	2	24	46	16	2	41.91	0.055
	42	—	1	21	67	18	3	42.01	0.045
	43	—	3	24	41	17	1	41.87	0.06
	44	—	1	18	43	14	1	41.95	0.05
	45	—	2	21	31	6	2	41.76	0.07
	46	—	—	13	23	10	—	41.93	0.07
	47	—	—	14	23	2	1	41.75	0.06
	48	—	1	16	16	7	—	41.72	0.08
1919_9 A....	49	—	4	6	14	—	—	41.42	0.10
	50	—	3	14	16	1	—	41.44	0.10
	51	—	2	14	9	2	—	41.41	0.095
	52	—	2	16	9	—	—	41.26	0.075
	53	—	2	6	6	1	—	41.40	0.14
	54	—	—	7	2	—	—	41.22	0.09
	55	—	1	2	2	—	—	41.20	0.225
	56	—	—	1	2	—	—	(41.67)	0.18
	57	—	—	—	1	—	—	(42.00)	—
	58	—	—	—	—	—	—	—	—
	59	—	—	—	—	—	—	—	—
	60	—	—	1	—	—	—	(41.00)	—

V

It has generally been taken for granted, as a basic assumption, that such differences as those here shown to hold between two successive year-classes, and between successive groups within a single year-class, are indicative of racial distinction. Obviously this assumption can not be maintained as wholly true. Moenkhaus (1895, 1898) indeed long ago demonstrated the occurrence of a significant annual variation within one race of fishes (in the case of the darters *Percina caprodes* and *Boleosoma*

nigrum). Schmidt (1921) has lately studied such annual fluctuations in great detail in *Zoarces*, and has induced like changes by experimental control of temperature in *Lebistes* (1919*a*, 1919*b*) and *Salmo* (1921). I have obtained similar experimental results for coregonine fishes and for *Esox lucius* (data yet unpublished).

On the other hand it has been clearly demonstrated in a number of cases that fine "racial" differences are inherited. Thus Schmidt (1917*a*, 1917*b*, 1918, 1920, 1921) has determined by his "offspring analyses" that a high degree of positive correlation holds between the number of segments and other features of the maternal parent and the unborn embryos of *Zoarces*. Similar results were obtained by Punnett (1904) for the viviparous shark, *Etmopterus* [*Spinax*] *niger*. In *Salmo*, Schmidt (1919*c*) has lately demonstrated that the finer differences in the number of vertebræ of both parents are inherited, and in the viviparous teleost *Lebistes reticulatus*, the same author has found (1919*a*, 1919*b*) that minor variations in the parental number of dorsal fin-rays are inherited. In somewhat similar fashion Sumner (1918, etc.) has demonstrated that subspecific differences in color and size in the mouse genus *Peromyscus* are inherited, even under changed enviromental conditions. A considerable body of indirect observational evidence might be brought forward, if needed, in confirmation of the assumption that these fine racial differences are inherited.

Clearly the same sort of variations as are induced by altered environmental conditions do characterize genetically distinct local races of fishes. Furthermore, these two sets of correlations display certain striking similarities or analogies, the significance of which the writer is attempting to determine in the series of studies of which the one here reported is a part.

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STUDIES ON FISH MIGRATION II. THE INFLUENCE OF SALINITY ON THE DISPERSAL OF FISHES[1]

DR. F. E. CHIDESTER,

West Virginia University, Morgantown, W. Va.

In connection with an extensive study of the factors influencing fish migration, certain experiments were performed during the summers of 1919 and 1920 to determine the effects of different salinities on the reactions of fish under laboratory conditions. Besides testing the animals with the salts of sea water, preliminary experiments were made with changed temperature and stream flow.

Material and Methods

The apparatus consisted of a two-tributary unit of a river system so arranged that different solutions could be introduced, affording the fish an opportunity to select the more favorable one. Two almost parallel troughs were so directed as to let the solutions flow down into a long receiving trough that had adjustable outlets in the middle.

There was also an intake at the extreme end of the large receiving trough so that if desired three intakes could be used. When only the two converging troughs were supplied with currents, a partition was placed across the middle of the receiving trough so that the water could flow laterally and eventually escape from the pool by the regular outlet.

The two tributary troughs were each 10 feet long, 4 inches deep and 4½ inches wide and the receiving trough was 10 feet long, 8 inches deep and 8¾ inches wide. The twin troughs were marked off in feet and conspicuously

[1] Contribution from the Biological Laboratory of the U. S. Bureau of Fisheries at Woods Hole, Mass.

labeled at the proper points so that from a single observation post, record could be taken of the distances traveled by fishes responding to the streams flowing down the incline.

Streams were introduced after temporary storage in two barrels located above the ends of the experimental troughs. In some experiments the inflowing currents came directly from the circulation pipes of the laboratory.

Experiments were performed with sea water, fresh water and combinations of the two, followed by tests with the individual salts of sea water in *m*/10 solutions. Temperature and stream flow were varied and proved most important adjuncts to the salts in affecting behavior.

In order to be quite certain that habit formation as a factor was eliminated, it was customary to select a trough used during the night for sea water inflow and introduce a substance less attractive, for the first few experiments with a group. As conditions of illumination were uniform and the troughs were so near each other, this procedure probably reduced the error due to a habit factor.

The fish were males, selected for apparent vigor and averaged about 12 centimeters in length. They were used for a complete series of experiments in lots of ten, then replaced by another ten of similar size. In the majority of the experiments, the species used was *Fundulus heteroclitus*. Its habits throughout the year were already known to the writer (1916, 1920). Loeb, Thomas and others had already studied its susceptibility to toxic substances. It is anadromous, highly resistant, yet furnishes quick reactions.

Fundulus majalis was used less frequently as it is not so resistant to laboratory conditions and behaves differently with reference to tides. The observations of Mast (1915) made it especially desirable to study the reactions to currents and accordingly a series of experiments was made.

Clupea harengus dies quickly in captivity. Its responses are extremely delicate and it has been used quite

successfully by Shelford, Powers and others in experiments on temperature, acidity, alkalinity and salinity. It proved too excitable for the experiments with which the present work was concerned.

Experiments

Fresh Water and Sea Water. (Temperature 20° C.) With apertures $\frac{5}{8}$ in. in diameter in two glass tubes directing horizontal streams of fresh water and sea water to a point six inches from the ends of the experimental troughs, it was found that 10 fish responded during 25 trials in such a manner that 11.8 was the value for responses to fresh water and 44.6 was the value for the sea water. These figures were obtained by multiplying the number of fish responding by the feet traveled up the trough towards the current, adding the total of 25 trials and securing averages for control and experiment.

The fish responded readily to the flow of water and since there was an admixture of fresh and salt water in the lower ends of the troughs, they did not at first discriminate the sea water before reaching a point 6 or 7 feet from the pool, that is 3 or 4 feet from the intake. As their reactions to the currents became established, however, they came in smaller numbers and finally became aligned along the sea water current at a distance of not more than a foot from the intake.

On changing the flow of fresh water to salt and vice versa, it was noted that at first the fish came into the trough formerly salt, and proceeded beyond the point where they usually traveled in fresh water. This was in part due to the habitual response and partly to the presence of some salts in the trough. On reaching the intake, they rapidly returned to the pool, one or two pioneered in each trough, then the whole group explored the salt trough and finally came to a point near the salt water intake.

Reactions to Salts in Solution

A preliminary series of experiments was run with fish immersed in *m*/10 solutions of the salts of sea water, made up in fresh water. Results were obtained similar to those recorded by Loeb, Thomas and others with fish and corresponding ones known to the writer from experiments with the larvæ of mosquitoes (1916).

By using the barrels above the experimental troughs solutions of the salts individually and in combination were introduced into the apparatus, with fresh water or sea water run as the control current. At first temperature and stream pressure were kept constant. The temperature averaged 20.5° C. and the pressure was sufficient to send the currents horizontally to a distance of six inches from the ⅝-in. glass tubes.

The reactions to individual salts as compared with fresh water are shown in the table below, only the averages at the end of 25 trials with 10 fish being recorded.

RESPONSES OF FISH TO SALTS

$MgSO_4$	46	Control, 0
$NaCl$	22.6	Control, 1
$CaCl_2$	6	Control, 20
$MgCl_2$	5.7	Control, 21.5
KCl	2	Control, 15

It is quite evident that with temperature and stream pressure the same, *Fundulus heteroclitus* will react quite definitely to salts. It is attracted to the less toxic ones, $MgSO_4$, and NaCl, and is repelled by those that are most toxic to it.

Similar experiments with sea-water solutions and sea water as the control current brought out quite clearly that for the species used, *m*/10 solutions of the more toxic individual salts were not strong enough to repel the fish. For example in the case of the most toxic, KCl, the score for 25 trials with 10 fish was 43 for the control sea water and 34 for the experimental current with KCl in *m*/10 solution.

Likewise, combinations of the salts showed only too

well the attractiveness of the mixed solutions. With an $m/10$ solution of $MgCl_2$ plus $MgSO_4$ and fresh water as control, the record was 11.2 for the control and 34.2 for the mixture. Again, in the case of KCl plus NaCl in $m/10$ solution, the score was 31 for the control and 17 for the mixture. With double sea water (specific gravity 1.050) and ordinary sea water at 20° C., it was found that the fish were attracted at the ordinary pressure and temperature, reacting to the stronger solution an average of 19.3 and to the control sea water 17.8 times. Further experiments should be run to determine the influence of antagonistic action of the salts in pairs. Whether or not the results will coincide with the results of permeability experiments will probably depend somewhat on the factor of temperature (Loeb and Wasteneys, 1912).

Influences other than Salts

The foregoing experiments indicate clearly that the behavior of the fish under consideration is materially affected by the salts with which they come in contact in fresh water. However, the factors involved in the *migration* of fish are by no means thus explained. It is worthy of note that the reactions of *Fundulus heteroclitus* to toxic salts or even sewage are dependent on temperature and stream pressure.

Temperature

Numerous experiments were tried with varying temperature and it was found that a temperature greater than 23° C. repelled the fish and caused them to align themselves along the current of fresh water at 20° C. in preference to the slightly warmer sea water.

With a reduced temperature, even one degree less than the control (19° C.), the fish were markedly attracted. In fact it was possible to lure them into double sea water, KCl or fresh water if these were presented at the proper temperature. Further experiments and observations are necessary for these and other species in order to determine the relation between gonad development, bodily condition and the responses to temperature change.

As pointed out by Gurley (1902), the minnows migrate to warming water for the purpose of spawning, while the cod and the salmon migrate to cooling water for the same purpose. Chamberlain believes that the salmon come into water warmer than the sea water (1906).

Field records for *Fundulus heteroclitus* secured by the writer in connection with another investigation (1916) indicate the importance of temperature. The fish began coming inland in the spring when the water was about 15° C. and continued to run in and out until the inland pools had reached a temperature in August of about 24° C. Then for a period of over two weeks, they ceased running. About September 1, when the temperature had again lowered, they appeared again and continued to run until the temperature ran down to 10° C.

Stream Pressure

When sea water was introduced through the $\frac{5}{8}$-in. glass tube with a force sending it horizontally to a distance of 6 inches, while fresh water was introduced through the experimental tube into the adjoining trough with a force sending it 12 inches from the end of the tube, there was no difficulty in luring the fish away into the fresh water and keeping them directed towards it.

Many experiments were made, toxic substances such as KCl and double sea water also being introduced, but the increased pressure always proved the powerful factor. Chamberlain (1906), Prince (1920) and others have previously shown that in the case of the salmon, migration into fresh water is delayed until the floods come down into the bays and small streams. The arrival of a volume of rushing water furnishes the needed stimulus and the fish proceed forthwith to obey their instinct to swim against the current.

That fish can determine the presence of toxic substances in sea water or in fresh water is unquestionably demonstrable. But we have much evidence that those fish lying offshore and habitually migrating up a certain

stream, will journey into polluted water, spawn in places where the eggs can not develop and in many cases, die in such water themselves.

Salmon are reputed to return to the lake-fed streams where they were spawned and there is considerable evidence that they are guided by temperature difference, probably also by the current pressure, number of waterfalls, oxygen content and even by food. There is no question (Meek, 1916), however, that salmon ascend streams where no salmon could hitherto have spawned.

The destruction of protecting forests, spoliation of natural waterways and the utilization of streams by manufacturers wishing to dispose of wastes are the factors which not only cause the death of fish embryos and adults, but prevent the natural control of insect pests by their destruction in the larval state.

Summary

1. *Fundulus heteroclitus* is able to discriminate toxic from non-toxic salts at a temperature and stream flow the same as the control.

2. Variations in temperature or in stream flow profoundly influence the reactions and are more powerful factors in the behavior of the fish than presence or absence of salinity.

3. In the apparatus used, errors due to the notable reactions of fish to currents of water have been reduced by presenting the control and experimental flows parallel to each other.

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SHORTER ARTICLES AND DISCUSSION

NOTE ON ASSORTATIVE MATING IN MAN WITH RESPECT TO HEAD SIZE AND HEAD FORM

ASSORTATIVE mating in man has been much discussed[1] but has been little investigated by scientific methods.

For characters such as age of husband and age of wife where there is an obvious preferential mating we may have coefficients of assortative mating as large as $r = + .75$. For stature, span and forearm Pearson has determined coefficients of about + .20 for span and span, + .20 for forearm and forearm, and + .28 for stature and stature in husbands and wives in his English series. The cross correlations for these various characters are in general smaller. For bodily characters other than stature the data are very few and are in general unsatisfactory.

With characteristic caution Pearson long ago suggested[2] that coefficients of assortative mating might be due to the husbands and wives being drawn from the same local races. The importance of this factor seems to be very small in his own materials.

This question must continually recur whenever assortative mating for physical characters is discussed. It seems very desirable, therefore, to obtain some measure of the correlation between husband and wife with respect to cephalic index, a character which has been considered of great importance by anthropologists in differentiating the races of Europe. For head size and head form we have had, as far as we are aware, until recently only the data for forty-eight families of Eastern European (Russian) Jews living in New York City, for which Boas[3] found assortative matings for cephalic index measured by $r = .15 \pm .10$.

Recently Frets in a series of papers[4] has given data for head

[1] The literature of the field has been reviewed up to 1912 by one of us: Harris, J. Arthur, "Assortative Mating of Man," *Popular Science Monthly*, 80: 476–492. 1912.

[2] Pearson, K., "Data for the Problem of Evolution in Man. III. On the Magnitude of Certain Coefficients of Correlation of Man," etc., *Proc. Roy. Soc.*, Vol. 66: 23–32. 1899.

[3] Boas, F., "Heredity in Head Form," *Amer. Anthrop.*, N. S., 5: 532. 1903.

[4] Frets, G. P., "Heredity of Head Form in Man," *Genetica*, 3: 193–400. 1921. This paper contains the original measurements. These have been to some extent checked against his other papers.

length, head breadth and cephalic index in a series of Dutch families. He has himself calculated a coefficient of correlation of .039 ± .034 for the cephalic index of husband and wife in 389 families.[5] We have felt it desirable to determine the correlation for length and width of head, as well as that for index.

Because of a suggestion by Pearson (*loc. cit.*) that the correlation apparently indicating assortative mating may be really due in some cases to an association of fertility with homogamy, we have thought it desirable to calculate all the coefficients of correlation in two ways: (1) by using the actual number of parents, and (2) by weighting the parents with the number of offspring indicated in Frets' tables.[6]

The correlation coefficients are as follows:

Length of husband's head and length of wife's head:

Parents only, $r = + .0487 \pm .0377$. $r/E_r = 1.29$.

Parents weighted with their children,

$r = + .0616 \pm .0376$. $r/E_r = 1.63$.

Breadth of husband's head and wife's head:

Parents only, $r = + .1197 \pm .0372$. $r/E_r = 3.22$.

Parents weighted with their children,

$r = + .1184 \pm .0372$. $r/E_r = 3.18$.

Index of husband's head and index of wife's head:

Parents only, $r = + .0231 \pm .0377$. $r/E_r = 0.61$.

Parents weighted with their children,

$r = - .0546 \pm .0376$. $r/E_r = 1.44$.

The constants are with one exception positive in sign. That for the breadth of husband and breadth of wife may perhaps be considered statistically significant in comparison with its probable error. The others, particularly that for the cephalic index, can not be so considered.

The coefficients may, therefore, indicate a slight assortative mating for the dimensions of the head. The coefficients, in common with those for physical characters other than stature, are relatively low. That the correlation for the cephalic index is so low is a point of particular interest. If cephalic index be a character of great importance in distinguishing races, and if correlations which have been demonstrated between the physi-

[5] Frets, G. P., "Erfelijkheid, correlatie en regressie," *Genetica*, 3: 1–27. 1921.

[6] We are able to abstract from Frets' tables 319 pairs of parents in which there were no indications of typographical errors when different tables were checked against each other. These had a total of 1328 recorded children. In calculating the probable errors of the coefficients we have used the unweighted number of parents as N.

cal characteristics of husband and wife be due primarily to the tendency to marry within the same racial group, one might expect a large correlation for cephalic index. Instead we find the lowest correlation of the three determined.

J. ARTHUR HARRIS,
ALBERT GOVAERTS

STATION FOR EXPERIMENTAL EVOLUTION,
COLD SPRING HARBOR, L. I.

A GYNANDROMORPH IN DROSOPHILA MELANOGASTER[1]

IN 1916 Hyde and Powell described a mosaic female with one eye eosin and the other blood. They interpreted this case in the light of Morgan's suggestion of 1914 that "Gynandromorphs and mosaics may arise through a mitotic dislocation of the sex chromosomes." In other words they believed one X chromosome carrying the gene for eosin went into the cells of one eye and the other X chromosome carrying the gene for blood went into the other eye. In 1919 Morgan and Bridges described a large number of gynandromorphs. The hypothesis of chromosomal elimination explains most of them, but a number of special cases are explained in other ways. One of their special cases was a male with one eye eosin and the other eosin vermilion. They explained this case by assuming that the egg had two nuclei, one of which after maturation had an eosin vermilion X chromosome and the other an eosin X chromosome. Further, they assumed each nucleus to have been fertilized by a Y sperm. These hypotheses would explain the facts that the individual was male throughout and that one eye was eosin vermilion and the other eosin.

In our experiments a somewhat similar mosaic appeared. The individual was made throughout, with one eye garnet and one white. The parentage was as follows: a garnet male was mated to a yellow white female. An F_1 wild-type daughter was mated to an F_1 yellow white male. From this pair of parents the mosaic arose. It was fertile and was bred to a garnet female. In F_1 all males and females were garnet. The F_1 garnet males and females were inbred. In F_2 the females were garnet but the males were garnet and white in approximately equal numbers (1,089 garnet to 1,026 white). This demonstrates very clearly that the mosaic was genetically a

[1] Zoological Laboratory Contribution No. 191. Indiana University.

garnet white. Professor Morgan writes us that he would also interpret this case on the binucleated egg hypothesis. We see clearly how the hypothesis may be applied and that the binucleated eggs described by Doncaster may give indirect evidence in its favor. Perhaps it is the best interpretation. We wish to point out, however, that there are other possibilities although they may have no direct or indirect morphological or experimental evidence in their favor.

Let us assume the individual started as a normal male, the single X chromosome carrying the genes for garnet and white. Since the mosaic did not carry the gene for yellow, the garnet white genes must have been brought together by a double crossing over in the mother. The only assumption we need to make is that during somatogenesis, the white end of one of the daughter X chromosomes became in some way inactive or lost. This would leave in one cell a whole X chromosome carrying white and garnet; in the other an imperfect X chromosome carrying garnet only. We know by test that white and garnet in the same chromosome give an eye practically indistinguishable from white. If one eye arose from the descendants of one of these two cells and the other eye from the second cell, we could account for the difference in color. The only assumption we need to make then is the loss or inactivation of the white gene in one of the early cleavage cells. On the binucleated egg hypothesis we must assume, first, the presence of two nuclei within the egg; secondly, that each nucleus is fertilized by a Y sperm; and thirdly, that the sex cells of the male arose from the descendants of only one of these nuclei, as all sperm were alike, carrying garnet and white.

A second possibility is that of somatic mutation. If the white gene in one of the cells should mutate to red, we would have a cell whose X chromosome carried the gene for garnet. If the descendants of this cell gave rise to one eye and the descendants of the other cells to the second, we would have one eye garnet and one garnet white, which is white. It is true that white eye has never reverted to red in all the thousands which have been bred. This fact renders this suggestion improbable but not impossible.

F. Payne,
Martha Denny

Zoological Laboratory,
Indiana University

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EXPERIMENTAL STUDIES ON THE DURATION OF LIFE

V. On the Influence of Certain Environmental Factors on Duration of Life in Drosophila [1]

PROFESSOR RAYMOND PEARL AND SYLVIA L. PARKER

A. Influence of Ventilation on Duration of Life

The standard method of handling *Drosophila* cultures, as described by Pearl and Parker (27), includes the plugging of the mouth of the bottle with absorbent cotton to prevent the escape of the flies. The theory of this practice, which is the custom in Morgan's laboratory, presumably is that air will pass in and out through the plug while the flies can not. No physicist or ventilation engineer would, we believe, accept this theory. Many years ago the senior author had occasion to make some observations on the ventilation of curtain-front poultry houses, and soon came to the conclusion that curtains of one thickness only, of the very porous jute bagging which is used for bran sacks, are practically nearly as effective in *preventing* the natural unforced circulation of air as a half-inch pine board would be. We may be sure that the plug of cotton used in *Drosophila* bottles will be an even more certain preventative of the natural unforced circulation of air. Theoretically one may perhaps hold that there is *more* circulation of air with a cotton plug than there would be with a cork stopper, but the difference must be infinitesimal.

[1] Papers from the Department of Biometry and Vital Statistics, School of Hygiene and Public Health, Johns Hopkins University, No. 67.

In the systematic survey which we are making, at the beginning of our experimental study of duration of life, it seemed desirable to test the influence upon this character of degree of natural ventilation of the bottles. It is the purpose of this first section of this paper to present the results of some experiments on this point.

Material and Methods

The experiments were carried out in two series. For the first, wild type flies of our Old Falmouth stock (Pearl and Parker (27)) belonging to Line 107, the duration of life constants of which have been given by Pearl and Parker (32), were used. These flies were of the 25th pedigreed generation. Eighteen mass matings of the flies of this line were started for the present experiments on March 13, 1922, and the flies to be used emerged March 23–30, 1922.

For the second series short-lived flies of Quintuple stock (Pearl and Parker (27)) were used, in the 27th pedigreed generation. Twenty-five mass matings of Quintuple line 405 were started April 10, 1922, and six mass matings of mixed Quintuple stock were started April 11, 1922. The flies for use in the experiment emerged April 22–27, 1922.

The procedure in making up the experiments was as follows: The flies were counted out each morning upon emergence into our standard one ounce screw top shell vials used in the determination of duration of life (Pearl and Parker (27)). Fifty flies were put in each bottle. The wild type flies were counted in through the counting tube described in these Studies, III (Pearl and Parker (44)). The Quintuple flies move through the tube so slowly, however, that there is time for moisture to condense and accumulate on the walls of the tube, killing some of the flies by drowning, and injuring others. Consequently the flies of this type were etherized and counted into the bottles. It has been shown in these Studies, III, that such etherization has no measurable

influence on duration of life. Each day's flies were divided equally between control and experimental groups. Fertility in the Quintuple flies is so low that even with the large number of matings the hatches on some days did not equal 100 flies. It thus resulted that there were a few bottles of the Quintuples with fewer than 50 flies to the bottle: 1 case with 40 flies in control and 40 in the experimental bottle, 1 with 37 flies in each bottle, and 1 with 23 flies in each bottle.

The *control* bottles were plugged with cotton in the ordinary way. The experimental, ventilated bottles were covered with one layer of silk bolting cloth of No. 48 mesh (48 meshes to the linear inch), this being the largest mesh which could be used without any possibility of a fly squeezing through the openings. The cloth was held firmly and evenly in place by an aluminum screw cap in the top of which a central hole a little more than ¾ inch in diameter had been punched out. This is practically the internal diameter of the shell vials which we use.

Both control and ventilated bottles were carried in 25° incubators, and all other procedure was that which has been described by the authors (27) as standard in the work of this laboratory on duration of life in *Drosophila*.

Results

The observed l_x distributions (survivors out of 1,000 starting together) for the wild type Old Falmouth Line 107 are given in Table I, together with the absolute numbers of flies on which the distributions are based.

These distributions of Table I are shown graphically in Fig. 1.

It is evident at once that the flies in the well-ventilated bottles outlive those in the ill-ventilated. Their expectation of life is greater at every age. The magnitude and significance of this difference can best be appreciated from the constants of duration of life set forth in Table II.

TABLE I

SURVIVORSHIP DISTRIBUTIONS (l_x) OF VENTILATED AND CONTROL FLIES
Old Falmouth, Line 107

Age in days	Number of survivors up to indicated age. Ventilated	Control
1	1,000	1,000
7	986	984
13	970	969
19	946	929
25	900	846
31	804	730
37	697	615
43	589	485
49	480	397
55	371	292
61	278	195
67	185	119
73	103	44
79	12	2
85	0	0
Absolute number of flies	946	931

TABLE II

FREQUENCY CONSTANTS OF d_x DISTRIBUTIONS
Wild Type, Line 107

Group	Mean	Standard Deviation	Coefficient of Variation
Control	43.66 ± .39	17.63 ± .28	40.38 ± .73
Ventilated	47.92 ± .40	18.22 ± .28	38.01 ± .67
Difference	+ 4.26 ± .56	+ .59 ± .39	− 2.37 ± .99

There is clearly a significant increase, amounting roughly to 10 per cent., in the mean duration of life, or expectation of life at emergence, in the flies in the ventilated as compared with the unventilated bottles, all other conditions both genetic and environmental having been the same in the two series.

That the increased amount of fresh air is the cause of the difference is evidenced by the behavior of the flies. In the ventilated bottles the flies tended at all times to congregate on the under side of the bolting cloth going down to the bottom for food occasionally, but otherwise exhibiting a strong preference for the region about the

mouth of the bottle, where the diffusion of air between inside and outside was going on most rapidly. This behavior is in no way characteristic, in our experience, of flies in the bottles stoppered with cotton. In those there

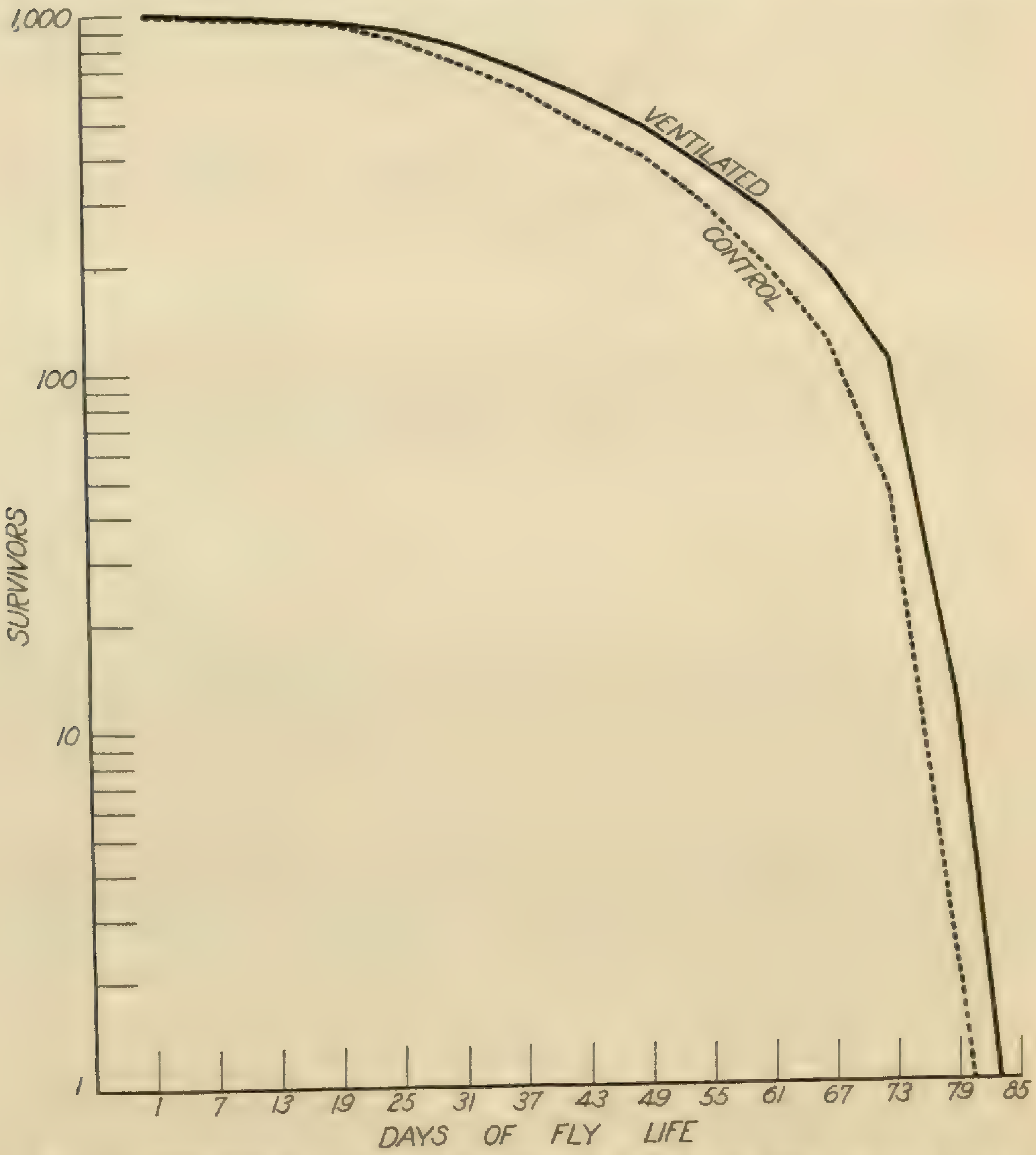

FIG. 1. Survivorship (l_x) lines for ventilated (solid line) and control (broken line) flies.

is generally a fairly even distribution of flies throughout the bottle, with such tendency towards concentration as there is, in the direction of the bottom near the food rather than the top.

In Table III are presented the survivorship distributions for the Quintuple flies. Because of their much shorter life span, as shown in the life tables of the first

one of the Studies (27), a shorter abscissal interval has been used in the grouping. The figures for Quintuple stock flies, and for an inbred Quintuple line (No. 405) are given separately.

TABLE III

SURVIVORSHIP DISTRIBUTIONS (l_x) OF VENTILATED AND CONTROL FLIES
Quintuple Stock and Line 405

Age in Days	Number of Survivors up to Indicated Age in			
	Stock Ventilated	Stock Control	Line 405 Ventilated	Line 405 Control
1	1,000	1,000	1,000	1,000
4	993	992	783	878
7	816	812	422	443
10	213	368	62	90
13	81	158	18	36
16	44	143	9	14
19	29	113	9	5
22	22	83	4	0
25	22	60	4	—
28	22	30	0	—
31	22	23	—	—
34	15	23	—	—
37	0	15	—	—
40	—	15	—	—
43	—	8	—	—
46	—	8	—	—
49	—	8	—	—
52	—	0	—	—
Absolute number of flies	136	133	209	221

The calculated constants from the d_x distributions are given in Table IV.

TABLE IV

FREQUENCY CONSTANTS OF d_x DISTRIBUTIONS
Quintuple Stock and Quintuple Line 405

Sort	Group	Mean	Standard Deviation	Coefficient of Variation
Quintuple stock	Control	11.07±.41	7.04±.29	63.58±3.54
	Ventilated	9.34±.26	4.57±.19	48.92±2.43
Difference		− 1.73±.49	−2.47±.35	−14.66±4.29
Quintuple (line 405)	Control	6.90±.13	2.90±.09	42.09±1.57
	Ventilated	6.78±.14	2.99±.10	44.06±1.71
Difference		− .12±.19	+ .09±.13	+ 1.97±2.32

The situation here is evidently quite different from what obtained with the wild type flies. The Quintuples lived somewhat longer in the control bottles than in the ventilated. In the case of flies from stock, the difference in the means amounts to 1.73 days, and is 3.5 times its probable error. The numbers are, however, small, and as an examination of Table III shows, the long survival of 2 individuals in the control series after age 34 accounts for a considerable part of the difference in the means. With a larger experimental sample much of the difference in the means would, we feel sure, disappear. The influence of these same two individual flies is clearly seen in the greatly increased variability of the control series over the ventilated in the stock groups.

In general we are of the opinion that in the case of Quintuple flies the difference in ventilation represented by a bolting cloth screen versus a cotton stopper has no significant influence upon duration of life. The results with extremely short-lived line 405, we regard as typical of what one should expect with Quintuple flies in this sort of an experiment.

The reason for the difference between wild type and Quintuple flies in their response to ventilation is founded, in our opinion, upon the normal differences in behavior between the two types. In Quintuples the wings do not function (the wing mutation in this stock is Vestigial). The consequence is that these flies are much less active, and generally appear to live on a lower metabolic plane, than wild type flies. Their oxygen needs are presumably smaller, and it would therefore be reasonable to expect that they would not show the difference in duration of life with increased ventilation that the wild type flies do. In this connection, it should be noted that their actual behavior in this experiment was in accordance with the view here suggested. They showed no such definite tendency to congregate at the top of the bottle under the bolting cloth as the wild type flies did. Their distribution was about the same in ventilated as in the control

bottles. Another consideration is that genetically Quintuple carries factors for very short life. These genes appear, in our experience with these flies, to be the overwhelmingly important factors in determining their length of life. No environmental factor, however favorable, makes much difference in their duration of life.

Summary

In experiments involving the determination of the duration of life in 2,576 individual flies, it has been found that in the case of *Drosophila* of wild type (*i.e.*, carrying no mutations so far as known), an increase of roughly 10 per cent. in the mean duration of life is brought about by increasing the ventilation of the culture bottles, by covering the mouth with one layer of No. 48 mesh bolting cloth, as compared with the use of cotton plug stoppers as is the usual practice in the culture of *Drosophila* in the laboratory. Owing, in our opinion, to fundamental differences in behavior, no such difference appears in the case of Quintuple flies.

B. Can the Duration of Life be Increased by Embryonic Juice?

If the theory of senescence and natural death which the senior author has developed in his "Biology of Death" (1–7) is true, one consequence of it should be that it might be possible to increase the duration of life, if by appropriate means one could restore the normal functional balance of the parts of the body after changes had set in with advancing age. Might it not be possible, by the use of X-rays for example, at the right stage of the life curve, and in proper dosage, to destroy cells, or perhaps even parts of tissues, which have got out of proper functional balance, and thus pave the way to their replacement by regeneration with fresh, "young" cells or tissues? In this way the life of the whole organism might be prolonged. The work of Frisch and Starlinger (45) with blood suggests that such a result might at least

be hoped for. In view of the known facts as to the potential immortality of tissue cells in cultures *in vitro,* and the apparent reason for the difference in the behavior of the same cells in respect of duration of life when they are in the multicellular body, all of which has been rather fully discussed by Pearl in the "Biology of Death" (*loc. cit.*), it would seem that this is a line of experimentation well worth following. We have a number of experiments along this line now in progress, particularly with X-rays, which we expect later to report upon. Some of the purely preliminary work has already been finished, and we wish in this paper to report one piece of it.

The brilliant researches of Carrel and his coworker Ebeling (*cf.* 46, 47) on the duration of life of cells in cultures *in vitro* have brought to light the extraordinarily interesting and presumably important fact that for the continued life of such cultures it is apparently essential to have in the culture medium a small amount of embryonic juice. In just what manner this functions is not yet clear, but the necessity of its presence seems well established.

It occurred to us in our preliminary work on prolongation of life in *Drosophila,* or as it is perhaps better to put it, on changing the form of the l_x line of the *Drosophila* life table, to see whether embryonic juice, applied at a point on the l_x curve after senescence had definitely set in, would have any effect upon the subsequent course of the curve, or in other words, upon the duration of life of the organism as a whole, comparable to its effect upon the life of cells in culture. The ideal way, of course, in such an experiment would be to get the embryonic juice to the tissues of the fly by a par-enteral route, but as no practical method of doing this occurred to us, we decided to feed it, and see if any results followed.

Material and Methods

The flies used in the experiment were all wild type, of Old Falmouth stock, and belonged to Line 107, pedigree

bred for 21 generations. The individuals for the experiment came from 20 mass matings of 3 pairs of parents each from this line. The bottles were started December 16, 1921, and the flies used in the experiment emerged December 28, 1921, to January 9, 1922.

The flies were counted through the counting tube into our standard shell-vials in groups of 50 each. Each day's bottles were divided into three groups at the beginning of the experiment, but all had the same regular treatment until the flies in them were 30 days old. This is a point where the l_x line is beginning distinctly to turn downward. From that time on until the end of their life one series of flies was given chicken juice in their food, and one series the juice and pulp of crushed *Drosophila* larvæ. The chick embryos used were 14 days old. The juice was extracted in a beef-juice extractor, and added to the regular food at the rate of approximately 2 c.c. to 100 c.c. of food. With the *Drosophila* larvæ, the whole pulp was used, and that too was added to the regular food at the rate of approximately 2 c.c. to each 100 c.c. of food. All the flies, experimental and control, were transferred every day to fresh food, made up that day, except on Sundays.

On Feb. 8 an accident happened to the incubator at the source of our chicken supply,[2] so that for 12 days no chickens were obtainable.

In all particulars except those specified above, the procedure in these experiments was the standard technique of this laboratory in duration of life work described in (27).

Results

The survivorship distributions are given in Table V, on the basis, *A*, of 1,000 starting at emergence, and, *B*, of 1,000 starting at age 31 days, that is at the time when the experimental feeding began.

[2] We are greatly indebted to our colleagues, Dr. and Mrs. Warren H. Lewis, for furnishing us with chicken material for this work.

TABLE V

SURVIVORSHIP DISTRIBUTIONS (l_x) OF GROUPS OF DROSOPHILA FED IN DIFFERENT WAYS

Old Falmouth Stock, Line 107

Age in Days	Number of Survivors up to Indicated Age in					
	Controls		Chicken Juice		Larval Pulp	
	A	*B*	*A*	*B*	*A*	*B*
1	1,000	—	1,000	—	1,000	—
7	975	—	970	—	970	—
13	945	—	935	—	930	—
19	884	—	873	—	858	—
25	797	—	784	—	719	—
31	616	1,000	648	1,000	591	1,000
37	491	796	456	703	464	785
43	362	588	313	484	283	479
49	286	465	246	380	210	356
55	212	345	198	306	156	264
61	156	253	152	234	116	196
67	120	197	110	170	82	140
73	59	96	58	89	46	78
79	21	34	24	38	28	48
85	7	11	9	14	3	5
91	1	2	0	0	1	2
97	1	2	—	—	0	0
103	0	0	—	—	—	—
Absolute number of flies	1,013	—	983	—	994	—

The biometric constants of duration of life calculated from the d_x distributions are given in Table VI.

TABLE VI

BIOMETRIC CONSTANTS FOR DURATION OF LIFE IN DROSOPHILA UNDER DIFFERENT CONDITIONS OF FOOD. *A*. FROM EMERGENCE. *B*. FROM AGE 31 DAYS ON

Class	Group	Mean (in days)	Standard Deviation (in days)	Coefficient of Variation
A	Control	39.60±.40	18.76±.28	47.38± .85
	Chicken juice	38.66±.40	18.61±.28	48.12± .89
	Larval pulp	36.74±.39	18.01±.27	49.03± .90
B	Control	19.72±.40	14.66±.28	74.35±1.62
	Chicken juice	17.51±.40	15.05±.27	85.96±2.06
	Larval pulp	17.12±.39	14.03±.28	81.98±1.94

It is evident that there are no large differences in mean duration of life between any of the groups. The l_x distributions and the constants are closely similar throughout. This is true whether the whole life is taken, or the expectation after age 31. The exact nature of the differences is shown in Table VII.

TABLE VII

DIFFERENCES IN MEANS OF TABLE VI

Class	Difference Taken	Value of Difference	Diff./P.E. Diff.
A	Control – chicken juice	.94 ± .56	1.66
	Control – larval pulp	2.86 ± .55	5.15
	Chicken juice – larval pulp	1.92 ± .56	3.46
B	Control – chicken juice	2.21 ± .56	3.93
	Control – larval pulp	2.60 ± .56	4.67
	Chicken juice – larval pulp	.39 ± .56	.69

The control group had slightly the greatest duration of life, both as a whole, and from the time of the beginning of the special feeding on. The flies fed larval pulp had the worst expectation of life, with those fed chicken juice in an intermediate position. None of the differences, however, is large. That some of them are significant statistically probably means no more than that the changed food is not quite so favorable for the flies as the normal, standard food. The numbers involved are large relatively, and the probable errors consequently small.

We must then conclude that the administration of embryonic juice in the manner, amount and time in the life cycle, which defined its administration in these experiments, does not bring about any prolongation of the life of the whole organism, comparable to its effect in tissue cultures *in vitro*. This does not necessarily mean that under other conditions of administration or dosage an effect in this sense might not be produced. We believe, however, that it is not probable that any prolongation of life can be brought about by this method, for the reason that in the first place the results of the present ex-

periment give no suggestion that with larger dosage any such result would appear, and in the second place, because the experiments of Bacot and Harden (48) indicate that as slight (or slighter) alterations of the food of *Drosophila* as those of the present experiments may produce marked effects in respect of viability.[3]

For some reason which we are unable to explain, the flies of Line 107 had, in all the series of this experiment, a lower mean duration of life than this line has ever shown before (*cf.* 32, 44, and section *A* of the present paper). The values are extremely even and consistent in this feeding experiment, but are about 10 days lower than what previous work has indicated as the normal duration of life in this line. There has been no other change in the line, in fertility or other characters. We are inclined to believe that the low values in the present experiments represent merely a temporary secular change (? seasonal) in the duration of life characteristic of the line.

Summary

In experiments involving the determination of the duration of life in 2,990 individual flies, it was found that there was no prolongation of the life of *Drosophila* produced by adding embryonic juice (either from the chick, or from the larvæ of *Drosophila* itself) daily to the food, to the amount of 2 per cent. of the total food material, beginning with the 31st day of the flies' life.

LITERATURE CITED

(The plan of numbering citations is explained in the second of these Studies, AMER. NAT., Vol. 56, p. 174.)

44. Pearl, R. and Parker, S. L. Experimental Studies on the Duration of Life. III. The Effect of Successive Etherizations on the Duration of Life of *Drosophila*. AMER. NAT., Vol. 56, pp. 273–280, 1922.

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[3] It ought, however, to be pointed out that the experiments of Bacot and Harden are extremely faulty from a technical standpoint. They evidently know little of the practical husbandry of *Drosophila*. Their cultures were incubated at 30° C. At this temperature one does not get anything remotely resembling normal physiological processes or duration of life, except after many months of acclimatization.

46. Carrel, A. and Ebeling, A. H. The Multiplication of Fibroblasts *in vitro*. *Jour. Exp. Med.*, Vol. 34, pp. 317–337, 1921.
47. *Id.* Heterogenic Serum, Age, and Multiplication of Fibroblasts. *Ibid.*, Vol. 35, pp. 17–38, 1922.
48. Bacot, A. W. and Harden, A. Vitamin Requirements of *Drosophila*. I. Vitamines B and C. *Biochem. Jour.*, Vol. 16, pp. 148–152, 1922.

VI. A Comparison of the Laws of Mortality in Drosophila and in Man

PROFESSOR RAYMOND PEARL

I

In the first of these Studies (27) there were presented for the first time, so far as I am aware, complete life tables for any other organism than man. Up to the present time there have been presented in the published results of the work of this laboratory on *Drosophila* (27, 32, 44, 49, 50) exact determinations of the duration of life in 24,329 individual flies. This is a statistically respectable mass of material, and warrants some general discussion.

In the first study a rough, purely graphical comparison of the l_x lines of the *Drosophila* and certain human life tables was instituted. This comparison, rough as is was, made apparent at once the fact that there was a fundamental similarity in laws of mortality in these two organisms.

It is my purpose in the present paper to make a more exact comparison of the values of the life table functions in the two cases. It will be seen that the similarity is even closer than was supposed from the rough comparison, and that in fact we are dealing here with qualitatively identical expressions of an obviously fundamental biological law.

II

Upon what basis shall any life table function, say l_x, of the *Drosophila* life table be compared with that of man? The life span of one of these organisms is best measured in days, while that of the other is measured in

years. This fact, however, offers no insuperable difficulty to the comparison. What is needed is to superimpose the two curves so that at least two *biologically equivalent* points coincide. The best two points would be the beginning and the end of the life span. But in the case of *Drosophila* our life tables start with the beginning of *imaginal* life only. The larval and pupal durations are omitted. In our preliminary comparison (27) we took human age 15, as the point corresponding biologically to the beginning of imaginal life in *Drosophila*.

I think we can get at this starting point more exactly by putting the human and *Drosophila* l_x curves together as a starting point at the age for each organism *where the instantaneous death rate* q_x *is a minimum.* In the case of *Drosophila,* I think we are safe in concluding, on the basis of the work of Loeb and Northrop (14–17) as well as from our own observations, that this point is at or very near the beginning of imaginal life. We shall accordingly take *Drosophila* age 1 day as this point. Our life tables show that certainly *after* this time q_x never again has so low a value. Indeed the fundamental law of mortality in *Drosophila* imagoes was stated in (27) in this way (p. 492): "the instantaneous death rate increases with age as a modified logarithmic function of x."

The latest edition of Glover's (51) United States Life Tables gives (p. 68) for white males in the original registration states the following values for q_x: for age 11–12 2.28, and for age 12–13, 2.29. We may, therefore, with sufficient accuracy take exactly 12 years as the minimum point, particularly as the l_x figures we shall have to use are tabled as of the *beginning* of the age interval.

For the other end of the life span we may conveniently take the age at which there is left but *one* survivor out of 1,000 starting at age 1 day for *Drosophila* and age 12 years for white males. This age for wild type *Droso-*

phila is, to the nearest whole figure, 97 days. To determine it for white males we have Table I, calculated from Glover's Table 9.

TABLE I

SURVIVORSHIP OF WHITE MALES IN ORIGINAL REGISTRATION STATES ON THE BASIS OF 1,000 AT AGE 12

Age	Number Alive at Beginning of Age Interval l_x	Age	Number Alive at Beginning of Age Interval l_x	Age	Number Alive at Beginning of Age Interval l_x
12–13.....	1,000	45–46	803	78–79	194
13–14.....	998	46–47	792	79–80	171
14–15.....	995	47–48	782	80–81	150
15–16.....	993	48–49	771	81–82	130
16–17.....	990	49–50	760	82–83	110
17–18.....	987	50–51	749	83–84	93
18–19.....	983	51–52	737	84–85	77
19–20.....	979	52–53	725	85–86	63
20–21.....	975	53–54	713	86–87	51
21–22.....	970	54–55	699	87–88	41
22–23.....	965	55–56	686	88–89	32
23–24.....	960	56–57	671	89–90	25
24–25.....	955	57–58	655	90–91	19
25–26.....	950	58–59	639	91–92	14
26–27.....	944	59–60	622	92–93	10
27–28.....	939	60–61	604	93–94	7
28–29.....	934	61–62	585	94–95	5
29–30.....	928	62–63	566	95–96	4
30–31.....	922	63–64	546	96–97	2
31–32.....	916	64–65	525	97–98	1.59
32–33.....	910	65–66	504	98–99	1.01
33–34.....	903	66–67	482	99–100	.63
34–35.....	896	67–68	459		
35–36.....	889	68–69	436		
36–37.....	881	69–70	412		
37–38.....	873	70–71	389		
38–39.....	865	71–72	364		
39–40.....	857	72–73	340		
40–41.....	849	73–74	315		
41–42.....	840	74–75	291		
42–43.....	831	75–76	266		
43–44.....	822	76–77	241		
44–45.....	812	77–78	217		

From this it appears that there is almost exactly one survivor at 98 years. So then we have as biologically equivalent life spans

97 *days* of *Drosophila* life as imago = 86 *years* of human life.

Whence it follows that

1 day of *Drosophila* life = .8866 year of human life and
1 year of human life = 1.1279 days of *Drosophila* life.

III

We are now in position to make an exact comparison between the life tables of the two organisms. This may be done perhaps most instructively by setting up l_x lines for the two forms on the basis of age in *centiles of the life span,* rather than days or years. That is to say, the whole comparable life spans (as defined in this paper) of 97 days in *Drosophila* and of 86 years for white males will each be divided into 100 equal parts, and the survivors at the attainment of the beginning of each centile interval will then be computed.

This is done for wild-type (long-winged) *Drosophila* males (Pearl and Parker (27) Life Table II) and male whites in original Registration states in 1910 (Glover's Table 9), in Table II.

The two life curves of Table II are shown graphically in Fig. 1, plotted on an arithlog grid. We have, in Table II and Fig. 1, for the first time, so far as I am aware, a precise quantitative comparison of the life spans and one of the mathematical functions of the mortality of two different organisms.

It will be noted that:

1. The form of the l_x distributions is fundamentally the same in both of these organisms over the equivalent life spans. Considering the extreme differences in habits of life, structure, physiology, and environmental stresses and strains in the two cases, this is a truly remarkable result. It seems to me to mean that the factors which determine individual longevity, and differences in this character, are biologically deeply rooted, at least as fundamental, apparently, as the factors which determine the specificity in the morphogenesis of organisms, and perhaps even more so. We are accustomed loosely to think that the prime factors in determining

TABLE II

SURVIVORSHIP DISTRIBUTIONS (l_x) FOR EACH CENTILE OF THE COMPARABLE LIFE SPANS OF (*a*) WILD TYPE DROSOPHILA MALES AND (*b*) WHITE MEN IN THE ORIGINAL REGISTRATION STATES IN 1910

Centile of Comparable Life Span	Numbers Alive at Beginning of Centile Age Interval		Centile of Comparable Life Span	Numbers Alive at Beginning of Centile Age Interval	
	Drosophila	Man		*Drosophila*	Man
0– 1.....	1,000	1,000	51–52	360	673
1– 2.....	991	998	52–53	344	659
2– 3.....	981	996	53–54	328	645
3– 4.....	972	994	54–55	312	631
4– 5.....	963	991	55–56	296	616
5– 6.....	954	989	56–57	280	601
6– 7.....	945	986	57–58	265	585
7– 8.....	935	983	58–59	250	568
8– 9.....	926	980	59–60	235	551
9–10.....	917	976	60–61	221	533
10–11.....	907	972	61–62	207	515
11–12.....	898	968	62–63	193	496
12–13.....	888	963	63–64	180	477
13–14.....	879	959	64–65	167	458
14–15.....	869	955	65–66	155	438
15–16.....	859	950	66–67	143	418
16–17.....	849	946	67–68	132	397
17–18.....	839	941	68–69	121	377
18–19.....	828	936	69–70	111	356
19–20.....	818	932	70–71	102	335
20–21.....	807	927	71–72	92	314
21–22.....	796	922	72–73	84	292
22–23.....	785	916	73–74	76	271
23–24.....	773	911	74–75	68	250
24–25.....	761	905	75–76	61	229
25–26.....	749	899	76–77	55	208
26–27.....	737	893	77–78	49	189
27–28.....	725	887	78–79	43	169
28–29.....	712	880	79–80	38	151
29–30.....	699	874	80–81	34	133
30–31.....	686	867	81–82	30	117
31–32.....	672	860	82–83	26	101
32–33.....	659	852	83–84	22	87
33–34.....	645	845	84–85	19	74
34–35.....	630	838	85–86	17	62
35–36.....	616	830	86–87	14	52
36–37.....	601	822	87–88	12	43
37–38.....	586	814	88–89	10	35
38–39.....	571	806	89–90	9	28
39–40.....	555	797	90–91	7	22
40–41.....	540	788	91–92	6	17
41–42.....	524	779	92–93	5	13
42–43.....	508	770	93–94	4	10
43–44.....	492	760	94–95	3.43	8
44–45.....	475	750	95–96	2.80	6
45–46.....	459	740	96–97	2.27	4
46–47.....	443	730	97–98	1.84	3
47–48.....	426	720	98–99	1.47	2.11
48–49.....	410	709	99–100	1.18	1.37
49–50.....	393	697	100	.94	.87
50–51.....	377	685			

human longevity are such things as the infectious diseases, exposure to unfavorable environment, etc. But *Drosophila,* which so far as is known has no infectious diseases, and in general meets a set of environmental

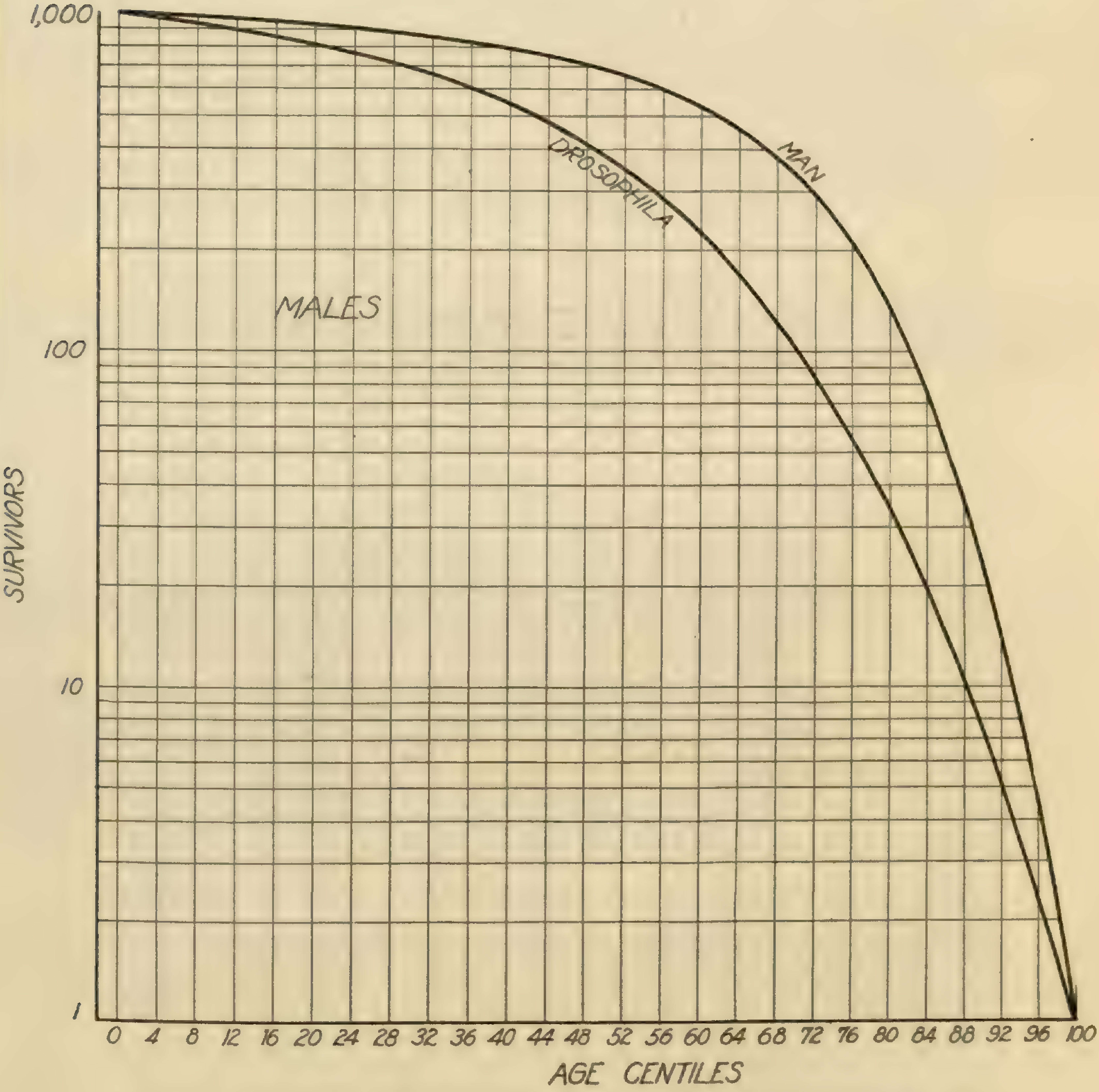

FIG. 1. Comparing the survivorship distributions of *Drosophila* and man (males in both cases) over the equivalent life spans.

conditions wholly different, both qualitatively and quantitatively, from those which operate on man, shows fundamentally the same form of distribution of degrees of longevity.

2. When compared exactly, on the basis of comparable life spans, the human being has at every equivalent age a higher relative expectation of life than does *Drosophila,* measured in terms of its own life span in each case. That this was the case for all but old age was concluded from the rough graphical comparisons of the first Study in this series. It is now seen that the same is true over the whole of life. From this fact the conclusion appears warranted that while the laws of mortality are fundamentally the same *in kind* for *Drosophila* and for man, they differ somewhat quantitatively. There is a temptation to conclude further that the quantitative difference finds its cause in man's own control and amelioration of his environment though sanitation and hygiene. Such a conclusion, however, seems to me not to be strictly warranted, in the light of our present knowledge. There is some suggestion that it is true, as was pointed out in the first of these Studies, from the fact that the progressive change of the human l_x curve in form during historical times has been in the direction of moving from the form typical of *Drosophila* to that now found for progressive, highly civilized groups of men. But definitive conclusions on the point must await further research.

3. The details of the quantitative differences in the two curves are interesting. When the first 25 per cent. of the equivalent life spans has been passed *Drosophila* has lost almost exactly 25 per cent. of the individuals starting life together, while man has lost but 10 per cent. When 50 per cent. of the life spans has been completed *Drosophila* has lost 72 per cent. of the individuals starting together, while man has lost but 31.5 per cent. At 75 per cent. of the life span, *Drosophila* has lost 94 per cent. of the individuals and man 77 per cent. From the 53d centile of the equivalent life spans on practically to the end, man has more than twice as many survivors out of a thousand starting together as does *Drosophila.*

Exactly similar results to those here presented are ob-

tained if one compares human and *Drosophila* life curves for females. Since nothing new in principle is brought out, it is not thought necessary to present the female curves here.

IV

In this paper it is shown that if we take as equivalent life spans in *Drosophila* and man the period between (*a*) the point in the life history of each organism where the specific death-rate (q_x) is a minimum, and (*b*) the point where there is one survivor out of 1,000 starting at the beginning as defined in (*a*), and then divide these equivalent life spans into 100 portions (thus measuring age not in absolute units but in centiles of the life span), the laws of mortality are fundamentally the same in kind in the two organisms. There is a quantitative difference expressible in the statement that at each centile age throughout the life span the number of survivors, out of the same original number starting together, is higher in man than in *Drosophila*.

In a subsequent paper, I hope to take up in detail the functional relations (in a mathematical sense) between the human and *Drosophila* equivalent l_x curves here presented.

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THE SYSTEMATIC LOCATION OF GENES BY MEANS OF CROSSOVER OBSERVATIONS

R. A. FISHER

Rothamsted Experimental Station

Introductory

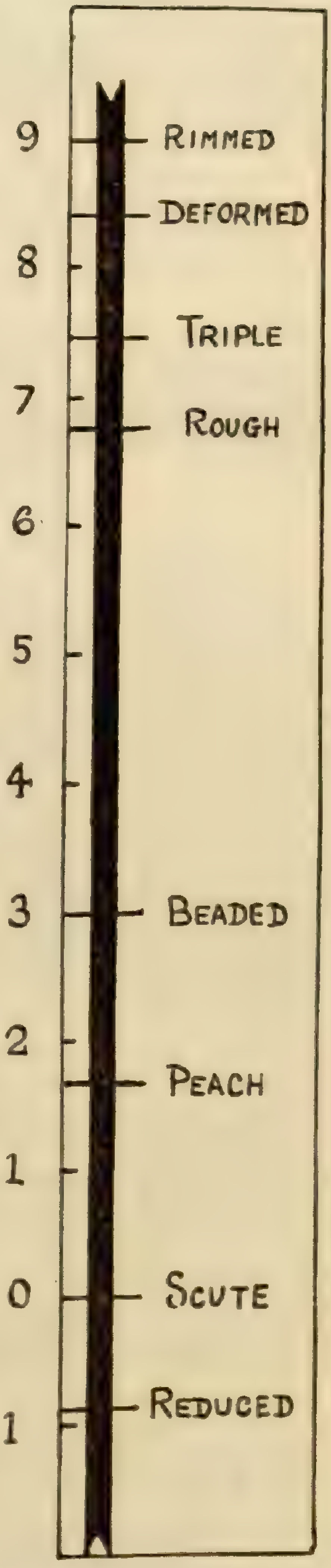
In the construction of a chromosome map, the distances between neighboring genes are equated to the percentage of crossovers which have been observed between them. Owing to errors of random sampling, and sometimes to other disturbing causes, inconsistencies always arise between the distances so determined. For example, in the important data given by Lancefield and Metz for the sex chromosome of *Drosophila willistoni* [1, p. 241] we have the following values:

TABLE I

	Crossover Percentage	Number of Observations	Number of Crossovers
Scute to Beaded	1.43	279	4
Beaded to Rough	2.42	455	11
Scute to Rough	7.09	6388	453

Within such a small range, double crossing over may be ignored; yet it would be wrong to use such inconsistencies as an argument against the linear arrangement of the genes. For although the true crossover values may be accurately additive, errors of random sampling will certainly disturb the observed percentages. The practical problem is to assign to the distances between the genes values which shall be as far as possible in accord with the whole of the observations available. In other words, we have to make use of as much as practicable, ideally the whole, of the information supplied by the data; giving due weight (i) to the greater accuracy of the values obtained from the larger number of observations, (ii) to the greater accuracy of values obtained from

closer pairs. In general, too, we shall have to consider not three genes only, but a large number, lying sufficiently close together for double crossing over to be ignored, the percentage observed between each pair of which gives indirect information as to the position of all the others.



In its general character the problem resembles those problems involving errors of observation, where a smaller number of unknowns are determined from a larger number of inconsistent equations, and which are usually solved by the method of least squares. The practical solution depends on the construction of a number of " normal equations " for the unknowns, in which the inconsistencies of the data are properly weighted and made to balance. To make the sum of the squares of the errors of the crossover percentages a minimum would, however, be wrong, and the method of least squares is not directly applicable. It has been shown that the whole of the information supplied by the data (2) is made use of by the method of maximum likelihood, and by a first approximation the required normal equations may be constructed.

2. Mathematical Theory

In the above example, if we write p_1 and p_2 for the two adjacent crossover ratios, the probability of the actual series of observations

will be proportional to

$$p_1^4(1-p_1)^{275}p_2^{11}(1-p_2)^{444}(p_1+p_2)^{453}(1-p_1-p_2)^{5935}$$

and the likelihood of any given pair of values for p_1 and p_2 will be proportional to the same quantity. In order to make this quantity a maximum for variations of p_1 and p_2, we have the equations

$$\frac{4}{p_1}-\frac{275}{1-p_1}+\frac{453}{p_1+p_2}-\frac{5935}{1-p_1-p_2}=0,$$
$$\frac{453}{p_1+p_2}-\frac{5935}{1-p_1-p_2}+\frac{11}{p_2}-\frac{444}{1-p_2}=0.$$

These equations are exact, but for practical purposes we need equations linear in p_1 and p_2, and a first approximation is sufficient; if p differs little from $x/(x+y)=x/n$, then

$$\frac{x}{p}-\frac{y}{1-p}=0-\left(\frac{x}{p^2}+\frac{y}{(1-p)^2}\right)\left(p-\frac{x}{n}\right)+\ldots=-\frac{n^3}{xy}p+\frac{n^2}{y}.$$

So that we may rewrite equations (1) in the practical and approximate form

$$\frac{279^3}{4\times 275}p_1+\frac{6388^3}{453\times 5935}(p_1+p_2)=\frac{279^2}{275}+\frac{6388^2}{5935},$$
$$\frac{6388^3}{453\times 5935}(p_1+p_2)+\frac{455^3}{11\times 444}p_2=\frac{6388^2}{5935}+\frac{455^2}{444}.$$

For each percentage observation, therefore, we have merely to calculate the two quantities n^3/xy and n^2/y; then normal equations may be constructed in the form

$$a_{11}p_1+a_{12}p_2+\cdots=b_1$$
$$a_{12}p_1+a_{22}p_2+\cdots=b_2$$
$$\cdots\cdots\cdots\cdots\cdots\cdots$$

where a_{12} is the sum of the quantities n^3/xy for which both p_1 and p_2 are involved, a_{11} the corresponding sum for all in which p_1 is involved, and b_1 the sum of the quantities n^2/y for which p_1 is involved.

3. Practical Example

In order to illustrate the practical application of this method to a complex case, we will consider the location of the 8 genes, from Reduced to Rimmed, in the middle

of the sex chromosome of *Drosophila willistoni*. We have here 7 intervals to determine, and fifteen crossover percentages are given [1]. Table II shows the data, and the series of weighting quantities derived from them.

TABLE II

	Percentage	x	n	n^2/y	n^2/xy	Unknowns Involved
Reduced–Scute.....	.95	27	2,848	2,875.26	303,287	p_1
Reduced–Rough....	6.24	37	593	632.46	10,136	p_1, p_2, p_3, p_4
Scute–Peach.......	1.81	8	442	450.15	24,871	p_2
Scute–Beaded......	1.43	4	279	283.06	19,742	p_2, p_3
Scute–Rough......	7.09	453	6,388	6,875.58	96,956	p_2, p_3, p_4
Scute–Deformed....	7.24	50	691	744.90	10,295	p_2, p_3, p_4, p_5, p_6
Scute–Rimmed.....	9.91	189	1,908	2,117.78	21,379	$p_2, p_3, p_4, p_5, p_6, p_7$
Peach–Beaded.....	1.70	3	176	179.05	10,504	p_3
Peach–Rough......	5.05	33	654	688.75	13,650	p_3, p_4
Beaded–Rough.....	2.42	11	455	466.27	19,287	p_4
Rough–Triple......	.49	4	809	813.02	164,433	p_5
Rough–Deformed ..	2.39	12	503	515.29	21,599	p_5, p_6
Rough–Rimmed....	2.26	62	2,742	2,805.43	124,072	p_5, p_6, p_7
Triple–Rimmed....	1.00	6	601	607.06	60,807	p_6, p_7
Deformed–Rimmed.	4.17	2	48	50.09	1,202	p_7

From this table we write down the normal equations

$$313{,}423p_1 + 10{,}136(p_2 + p_3 + p_4) = 3{,}507.72$$

$$10{,}136p_1 + 183{,}380p_2 + 158{,}509p_3 + 138{,}766p_4 + 31{,}674p_5 + 31{,}674p_6 + 21{,}379p_7 = 11{,}103.93$$

$$10{,}136p_1 + 158{,}509p_2 + 182{,}663p_3 + 152{,}416p_4 + 31{,}674p_5 + 31{,}674p_6 + 21{,}379p_7 = 11{,}521.58$$

$$10{,}136p_1 + 138{,}766p_2 + 152{,}416p_3 + 171{,}703p_4 + 31{,}674p_5 + 31{,}674p_6 + 21{,}379p_7 = 11{,}525.74$$

$$31{,}674\ (p_2 + p_3 + p_4) + 341{,}778p_5 + 177{,}345p_6 + 145{,}451p_7 = 6{,}996.42$$

$$31{,}674\ (p_2 + p_3 + p_4) + 177{,}345p_5 + 238{,}152p_6 + 206{,}258p_7 = 6{,}790.46$$

$$21{,}379\ (p_2 + p_3 + p_4) + 145{,}451p_5 + 206{,}258p_6 + 217{,}460p_7 = 5{,}580.36$$

Using a calculating machine, the work so far is rapid and mechanical; the solution of the normal equations may in this case be much simplified by observing the uniformity of some of the sets of coefficients, a type of uniformity which is probably characteristic of crossover data. Thus by considering $(p_2 + p_3 + p_4)$ as a single quantity, p_1 is immediately expressible in terms of it, and by solving the last three equations we may do the same for p_5, p_6 and p_7; substituting finally in equations (2, 3,

4) we solve them for p_2, p_3 and p_4, and obtain the values shown in Table III.

The seven values obtained give mutually consistent values for the crossover percentages between the fifteen pairs tested, and are therefore suitable for the construction of chromosome map. If the conditions of Maximum Likelihood had been exactly fulfilled they would agree better than any other consistent series of values with the percentages observed. As it is, it is only in the aberrant value of p_7 that the assumption that the observed values are approximately correct breaks down, and it is probable that such cases will only occur when the data are admittedly insufficient.

TABLE III

	Calculated	Observed	Difference d	Standard Error σ	$\frac{d^2}{\sigma^2}$
Reduced–Scute	.90 p_1	.95	+ .05	.18	.08
Reduced–Rough	7.66	6.24	−1.42	1.09	1.70
Scute–Peach	1.67 p_2	1.81	+ .14	.61	.05
Scute–Beaded	2.98	1.43	−1.53	1.02	2.31
Scute–Rough	6.76	7.09	+ .33	.31	1.13
Scute–Deformed	8.40	7.24	−1.16	1.06	1.20
Scute–Rimmed	8.97	9.91	+ .94	.65	2.09
Peach–Beaded	1.31 p_3	1.70	− .39	.86	.21
Peach–Rough	5.09	5.05	− .04	.86	.00
Beaded–Rough	3.78 p_4	2.42	−1.36	.89	2.34
Rough–Triple	.69 p_5	.49	− .20	.29	.48
Rough–Deformed	1.64	2.39	+ .75	.57	1.73
Rough–Rimmed	2.21	2.26	− .05	.28	.03
Triple–Rimmed	1.52	1.00	− .52	.50	1.08
Deformed–Rimmed	.57 p_7	4.17	+3.60	1.09	10.91
				$\chi^2 = 25.34$	

Table III is arranged to compare the differences between the calculated and the observed percentages with the standard errors due to sampling; except for p_7 all the differences are less than twice their standard errors; thus showing the general agreement between the data and the theory of linear arrangement of the genes. The fit, however, is not a close one, even if we omit p_7; in the present state of our knowledge this will not throw any

doubt on the scheme of linear arrangement, but will suggest that the crossover ratios in this part of the chromosome were not constant in all the strains used to compile the data.

In estimating the Goodness of Fit of data of this kind, χ^2 may be calculated by summing the values of d^2/σ^2, as in Table III Attention should, however, be called to the fact that it has been recently shown (3) that in entering Elderton's Table we must put n' equal to one more than the number of degrees of freedom, remaining after we have fitted our unknowns to the data. In the present case we have found 7 unknowns from 15 equations, leaving 8 degrees of freedom, so that n' should be 9, and not 16.

In conclusion it should be noted that to be available for the use of this process the crossover data should be stated in the form in which it is given by Lancefield and Metz, in which the crossovers tabled between any two genes do not include those experiments in which an intermediate gene was under observation. The practice of throwing together all the crossovers between two genes, in order to improve the ratios between the more distant points, causes the same crossover to appear repeatedly in different entries. The data are no longer the product of independent experiments, and must be re-summarized before reduction.

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LINKAGE IN PEROMYSCUS

DR. F. B. SUMNER

THE SCRIPPS INSTITUTION FOR BIOLOGICAL RESEARCH, LA JOLLA, CALIF.

STUDENTS of Mendelism are beginning to display the same interest in possible homologies between the genetic factors or "genes" of different species of animals or plants which the morphologists of thirty years or more ago did in homologies between organs. In considering a given case of suspected homology between genes, two criteria are, so far as I know, employed: (1) Resemblance between the developed characters which are attributed to the action of supposedly homologous genes. Mere similarity of appearance, however, is recognized as an extremely fallible criterion of homology here as in the case of comparative anatomy. (2) Agreement between the "cross-over" value shown by a pair of linked factors in one species, as compared with the corresponding value shown by supposedly homologous factors in another species. If both of the two linked genes under consideration are found to have much the same somatic effects in the two species, and if, furthermore, the degree of linkage is approximately the same in the two cases, the argument is strong for a twofold homology.

Metz[1] and Sturtevant[2] have been investigating the parallel mutations of several species of *Drosophila,* and it is not unlikely that this genus will furnish the best material for the study of genic homologies, just as it has shown incomparable superiority for certain other lines of genetic research.

For rodents, what appear to be parallel mutations have been shown to occur among numerous species, even ones

[1] *Genetics,* March, 1918.

[2] *Genetics,* January, 1921.

belonging to widely different families.[3] In one case, that of the mutation known as "pink-eye," not only is the visible modification closely similar in rats and mice, but the linkage relations between this factor and that for albinism are known to be of the same order of magnitude in the two animals.[4]

Some years ago, Castle[5] described two similar mutations in the Norway rat, which he termed "pink-eyed yellow" and "red-eyed yellow," respectively. These, according to the published descriptions, differ chiefly in the color of the eyes, the latter variety having darker eyes than the former. These two mutations, and likewise true albinism, were all found to result from the modification of distinct genetic factors. Any two of them, when crossed, gave rise to the wild type in the first hybrid generation. On the other hand, further breeding tests led Castle to conclude that all three of these factors were linked. When red-eyed and pink-eyed rats were interbred, the cross-over percentage proved to be about 18. When pink-eyed rats were crossed with albinos, this value proved to be about 21. On the other hand, the linkage between red-eye and albino proved to be almost absolute. One hundred and sixty F_2 albinos and 57 F_2 red-eyed yellows, when mated with pure red-eyes and albinos, respectively, yielded but a single offspring which was not of the wild type.

More recently Dunn[6] has tested the linkage between this same red-eyed condition and albinism in the rat. From his own data he computes a cross-over value of 1.8 per cent., but when his data are combined with those of Castle, this value falls to less than one per cent.

Castle and Dunn have likewise tested the degree of linkage between "pink-eye" and albinism in the mouse

[3] Dunn has compiled these cases in a useful article in the *Journal of Mammalogy*, August, 1921.

[4] According to Castle, the percentage of cross-overs is 21 for rats and 14 for mice. This may or may not be construed as evidence of "homology."

[5] American Naturalist, February, 1914; *Science*, August 6, 1916 (with Wright); Carnegie Institution Publications 241 and 288.

[6] *Genetics*, May, 1920.

(*Mus musculus*). The proportion of cross-overs was found to be about 14 per cent.

Some five years ago I described a pale, red-eyed mutant of *Peromyscus*,[7] which originated among the offspring of three sibs in the F_2 generation of a cross between *P. maniculatus rubidus* and *P. m. sonoriensis*. Since I have already described this "mutant" race rather fully, and since it will again be discussed shortly in a paper by Mr. H. H. Collins and myself, I need not enter into a detailed account of it here. I have not seen specimens of the "red-eyed yellow" rats described by Castle, but I find little in the description of that race which is at all at variance with my own "pallid" race of *Peromyscus*. The latter has undergone a great reduction of the black pigment, while the yellow pigment has been little if any affected. The eyes are commonly dark red, rather than pink, though they present a considerable degree of variability, ranging from a condition not much darker than the true pink of albinos to a condition not much paler than the normal. There are, however, no real intergrades between the pallid mice and the wild type, and the behavior of this complex of characters in crosses is that of a simple monohybrid recessive. Furthermore, it is not an allelomorph of albinism, since the wild type alone results from matings between albinos and pallids.

I have recently carried out tests of the linkage relations between this factor and that for albinism.[8] Thus far, it has not been found practicable to devote any considerable proportion of my time to this phase of the subject, and the numbers are accordingly inadequate for any exact measurement of cross-over values. They are, none the less, sufficient to show the existence of a high degree of linkage between these factors. The number

[7] *Genetics*, May, 1917; AMERICAN NATURALIST, August–September, 1918. This mutant was at first referred to as a "partial albino"; later the noncommittal term "pallid" was applied to it.

[8] The albinos used were all derived from a single brood belonging to the subspecies *Peromyscus maniculatus gambeli*.

of F_2 individuals derived from simple F_1 x F_1 matings is too small to give a representative dihybrid ratio. The really important tests have been made with "extracted" albinos and pallids of the F_2 generation.

Matings have been made (1) between "extracted" albinos and "pure" pallids (*i.e.*, those known to be free from the factor for albinism), (2) between extracted pallids and pure albinos, and (3) between extracted pallids and extracted albinos. There were likewise a number of matings in which the pedigrees were less simple.[9] On the assumption of a wholly independent segregation of these factors, our F_2 pallids (of simple pedigree) should have a 2/3 chance of being heterozygous for albinism, while our F_2 albinos should have a 3/4 chance of being either homozygous or heterozygous for pallid.[10]

Eighteen F_2 mice were involved in these tests. The total number of offspring derived from these was 135, the number per parent ranging from 3 to 26. By no means all of these parents, taken singly, have thus far given birth to a sufficient number of young to prove their genetic composition with any certainty. But the cumulative testimony of all of these matings is overwhelming. Not a single pallid mouse and only two albinos have appeared among the 135 young which have thus far been born. Had there been a normal proportion of "carriers" among the parents, these matings should have yielded 37 albinos and 18 pallids, as the most probable "expected" numbers. That all of the offspring with two exceptions (these being sibs) were of the wild type is evidence of a high degree of linkage (in this case "repulsion") between the albino and the pallid factors.[11]

[9] Back-crosses and heterozygous albinos figured in some of these pedigrees. In these cases the odds are different from those which hold for individuals derived from the simpler types of mating. They have, however, been computed for every animal used. In about half of the "extracted" albinos, for example, there was only a 5/8 chance that the individual carried the pallid factor.

[10] It is a safe assumption that the double recessive form would be albino.

[11] It might be supposed that the testimony of 18 parent mice, even if all of these were shown conclusively to be lacking in "cross-over" gametes,

From these considerations we may regard it as not unlikely that my "pallid" race of *Peromyscus* has resulted from the mutation of a genetic factor homologous with that which has mutated in the case of Castle's "red-eyed yellow" rats.

This decisive result, as regards the existence of linkage between the pallid and albino factors in *Peromyscus,* stands in contrast with the apparent absence of such linkage in another cross between mutant strains of these mice. Albinos were mated with mice belonging to a strain which I have elsewhere referred to rather inappropriately as "yellows."[12] The latter vary from clay color to a distinctly reddish hue, according to the strain, and are characterized primarily by a marked increase in the length of the "agouti" cross-band and by a decrease in the proportionate number of all-black (unbanded) hairs in the pelage. Where present, however, the black pigment is of full intensity. This applies to the basal zone of the body hairs, both dorsal and ventral, to the black hairs of the dorsal tail stripe, as well as to the eyes, ears and soles of the feet.

Matings between albinos and "yellows" have resulted exclusively in F_1 mice of the wild type (dark). An F_2 generation of 83 was obtained, consisting of 52 dark individuals, 13 yellows and 18 albinos. On the assumption of purely random assortment of gametes, the "expected" numbers are 44, 15 and 20, respectively. The observed numbers are doubtless within the range of "accidental" variability. In any case they give no evidence

would not be sufficient to prove the existence of linkage. It should be repeated, however, that we are not here dealing with cases in which there would be merely an equal chance of combining the two mutant factors in the same individual. The odds in favor of this (linkage aside) may, as stated above, be as high as 2 to 1, or even 3 to 1. Thus, the likelihood of obtaining, by chance alone, 17 non-cross-over cases out of 18 becomes vanishingly small.

[12] *Genetics,* May, 1917; AMERICAN NATURALIST, August–September, 1918. A more complete account of these mice, dealing with two subvarieties differing somewhat in color, is included in a forthcoming paper by Mr. H. H. Collins and myself.

of linkage, the occurrence of which would have reduced the proportionate number of dark individuals, instead of increasing it.

The number of F_2 albinos and yellows which have been thus far tested is very small, but it is of interest that the proportion of recombinations is even greater than would be expected from random assortment. Inclusion of these meager results in the present report seems justified by the probability that we shall not soon rear any considerable number of hybrids between the yellow and albino varieties.

Seven extracted albinos have been mated with pure yellows. Three of these have given only yellow offspring, the numbers being 9, 13 and 21, respectively. Thus, three of these seven albinos are, in all probability, double recessives (*ccyy*). (One in four should be double recessives, according to chance.) Three other albinos have given mixed offspring. They are evidently of the formula *ccYy*. The remaining one appears to have the formula *ccYY*, as judged by the production of 15 dark young.

Two extracted yellow females mated with a (supposedly) pure albino male gave birth to 4 albinos and 4 dark.[13] No albinos would be expected here if linkage were complete, while only one third should be albinos in the total absence of linkage. Thus the number of recombinations is again too high, even on the assumption of no linkage.

These numbers are, of course, very small. But even here such proportions would have been quite improbable had any marked degree of linkage existed—such, for example, as has been found to exist between the pallid and albino factors.

[13] It is only fair to add that 4 yellows likewise resulted from these matings. This was doubtless due to the fact, unsuspected at the time, that the albino male carried the "yellow" factor, one of his *two* great-grandparents having been heterozygous for yellow.

THE SOUND-TRANSMITTING APPARATUS OF SALAMANDERS AND THE PHYLOGENY OF THE CAUDATA

E. R. DUNN

SMITH COLLEGE

RESEARCHES by Kingsbury and Reed, extending through a number of years, have shown that the sound-transmitting apparatus of salamanders consists of two elements. These are the columella and the operculum.

In the most recent paper on this subject, Reed (1920) gives a résumé of all the previous work, an extensive account of the state of affairs in the *Plethodontidæ,* a brief account of the conditions in other forms, and the findings are presented in the form of a family tree.

The purpose of the present article is to add an account of the condition of the apparatus in two forms not seen by Reed, to question the condition described by Kingsbury and Reed for *Dicamptodon ensatus* (*Ambystoma tenebrosum* Auct.), to suggest a somewhat different interpretation of the facts observed by them, and to propose a somewhat different phylogeny, which seems to agree quite as well with the otic apparatus and far better with other anatomical features.

Kingsbury and Reed (1909) were unable to examine any of the Asiatic forms related to *Hynobius.* These forms, as Cope pointed out long ago, are rather different from the *Ambystomidæ,* with which they have usually been associated, and should in fact form a family *Hynobiidæ.*

I have recently been able to examine large series of *Hynobius leechii* from Korea. This animal shows a condition of the otic apparatus different from any seen by Kingsbury and Reed, and a condition which I am compelled to consider primitive. Both columella and oper-

culum are present as free and distinct elements. Both are readily movable. There is a m. opercularis.

I have not been able to examine skulls of *Onychodactylus,* or of *Ranodon.* Okajima's (1908) figures of *Onychodactylus* show only one element which is in appearance much like that of *Cryptobranchus.* This is very different from the appearance of the apparatus of *Hynobius.* It is evident that either fusion of operculum and columella has taken place or that the operculum has not developed. *Onychodactylus* is partly aquatic, a mountain brook animal. *Cryptobranchus,* which, as I shall show later, is a derivative of the *Hynobiidæ,* has failed to develop the operculum. Probably the same is true of *Onychodactylus* and of *Ranodon* as well, although for the latter Wiedersheim's (1877) figure is all we have. Still, as Kingsbury and Reed (1909) say, his Fig. 67 "suggests a condition such as is found in *Cryptobranchus.*"

Rhyacotriton olympicus was not examined by Kingsbury or by Reed. This animal (Dunn, 1920) possesses both columella and operculum. The columella is free from the periotic and is readily movable. The operculum is little developed. The animal is in part aquatic, a mountain brook species.

Dicamptodon ensatus was examined by Kingsbury and Reed (1909), and while my dissection of an adult showed the state of affairs which they describe, I can not follow them in calling it "much like that in the adult *Ambystoma.*" In adult *Ambystoma* the columella is solidly fused to the periotic. A bony operculum nearly fills the opening of the fenestra, and is attached by a membrane around its circumference. In *Dicamptodon,* on the other hand, about half of the fenestra is filled by the plate of the columella, and the remainder by cartilage. The cartilage extends around the plate of the columella. There is nothing that could be called a definite operculum. If the cartilage is called the operculum, then the columella and operculum are fused and the operculum is fused to

the ear capsule by nearly its whole border. It seems to me that in this case the columella is at least more free than in *Ambystoma,* and the operculum less developed. This would be in line with what is known of the habits of *Dicamptodon.* It is a much more aquatic animal than is *Ambystoma.*

In the Caudate sound-transmitting apparatus, taking Reed (1920) as a basis, there are the following sets of conditions:

I. Both columella and operculum present. Both free.
Hynobius, Rhyacotriton.

II. Operculum not developed. Columella free.
Cryptobranchus.
Megalobatrachus Ranodon ?,
Onychodactylus ?, *Dicamptodon* ?

III. Operculum developed, free. Columella fused to periotic, stylus present.
Salamandra, Ambystoma.

IV. Operculum developed, free. Columella fused to periotic. Stylus absent.
Triturus, Pachytriton, Pleurodeles ?,
Tylototriton ?.

V. Operculum developed, free. Columella ?.
Siren, Batrachoseps.

VI. Both columella and operculum present, fused together, free from periotic.
Necturus.

VII. Both columella and operculum present. Fused together. Operculum attached by narrow fusion to periotic.
Amphiuma, Plethodontidæ
(exc. *Batrachoseps*).

Inasmuch as II is a condition found also in larvæ, there is no reason to suppose that the animals in which this condition occurs form a natural group.

Condition V has been commented upon by Reed (1920), and I am fully in accord with his ideas in this connection. *Siren* and *Batrachoseps* are certainly not related. Both are extremely specialized. *Batrachoseps* has certainly passed through stage VII. *Siren* has certainly passed through an ancestral period of terrestrial life, yet its other peculiarities are such that it is dangerous to state that its relationships are with the forms in stage IV.

The forms which show condition VI and condition VII

form what Reed (1920) calls Legion II, as distinct from the forms which show conditions I–V (exc. *Batrachoseps*), which Reed calls Legion I.

But the sound-transmitting apparatus of *Necturus* agrees with that of *Amphiuma* and the *Plethodontidæ* only in having the columella and operculum fused. There is no reason to suppose that such a fusion may not have occurred twice, especially as the details of the fusion in *Necturus* differ somewhat from the manner in which the fusion occurs in *Amphiuma* and the *Plethodontidæ*. In *Necturus* the columella forms a goodly part of the plate-like portion of the apparatus. In the forms of condition VII, the plate-like portion is almost entirely composed of the operculum, and the columella is represented by the stylus. In this case the evidence of the ear bones is non-committal. Considered apart from all other features of the anatomy condition VII might equally well be derived from condition VI or both independently from condition I. But, as we shall see, evidence from other features of the anatomy precludes our regarding *Necturus* as intervening between the *Plethodontidæ* and the other Mutabilian forms.

It is extremely interesting to note that Reed has found almost exactly the same state of affairs in *Amphiuma* and in the *Plethodontidæ*. The exact relationships of *Amphiuma* have long been in dispute, and while I prefer to be conservative about the position of the animal, I think it extremely likely that further evidence will show that it is closer to the *Plethodontidæ* than it was placed in the older classifications.

Any classification should be based upon all available characters, so that possible parallelisms will not lead to wrong conclusions. In the present instance we are dealing with a stock neither absolutely terrestrial nor absolutely aquatic. From this stock there have been several branches which have become more aquatic and several which have become more terrestrial. Excellent examples of this are the numerous incursions into a mountain

brook habitat, with the penalty of loss or reduction of lungs. The list is extensive, *Onychodactylus*, *Rhyacotriton,* four species of *Triturus, Salamandrina, Chioglossa,* all the stock of the *Plethodontidæ,* an assemblage representing four families. The sound-transmitting apparatus is admittedly correlated with the mode of life. Therefore as a character in determining relationships it must be used with extreme caution.

The following outline classification of salamanders does not counter any of the facts concerning the otic apparatus, and is based on many characters.

As regards the *Plethodontidæ* and the *Hynobiidæ,* revisions of both are nearly completed, based on the examination of some 8,000 specimens of the first family and 1,000 of the second.

The *Sirenidæ* are the most isolated group. Scarcely a character can be found to ally them with one or another of the main stocks. The pelvis is gone, the skull is that of a very specialized larva, the hyoids are those of almost any larva, the tail vertebræ are very different from those of any other salamander, inasmuch as there is no hæmal arch. There are flat plates on each side which do not meet in the mid-ventral line. There is no prearticular.

The *Proteidæ* are only slightly less isolated. The pelvis differs in having an anterior median projection and no ypsiloid apparatus. The skull is larval. The branchial arches are reduced from the primitive larval quota. The prearticular is absent.

The *Amphiumidæ* also have modified larval branchial arches, and the pelvic girdle lacks the ypsiloid apparatus. But *Amphiuma* has an adult skull which resembles remotely that of the *Salamandridæ.* The otic apparatus is that of the *Plethodontidæ.* There is no prearticular bone. It is quite possible that this genus is descended from primitive Salamandrids.

The others have directly comparable skulls, branchial arches, and pelves, and in dealing with their relationships we are on much firmer ground.

Several characters divide them into two series, which should, I think, rank as superfamilies.

1. Prearticular bone. Present in *Cryptobranchidæ* and *Hynobiidæ*, and absent in *Ambystomidæ*, *Salamandridæ* and *Plethodontidæ*.
2. Second epibranchial. Present in *Cryptobranchidæ* and *Hynobiidæ*, and absent in *Ambystomidæ*, *Salamandridæ*, and *Plethodontidæ*.
3. First ceratobranchial and first epibranchial fused into a single cartilaginous rod in *Cryptobranchidæ* and in *Hynobiidæ*. Separate elements in *Ambystomidæ*, *Salamandridæ* (exc. *Salamandra*, where all parts fuse), and *Plethodontidæ*.
4. Nasals meeting in median line and premaxillæ without nasal process in *Cryptobranchidæ* and *Hynobiidæ*. Nasals separated by nasal spines of premaxillæ in *Ambystomidæ*, *Salamandridæ*, and *Plethodontidæ* (exc. *Pseudotriton*, where nasals overlap premaxillary spines).
5. Pubotibialis muscle fused with puboischiotibialis in *Cryptobranchidæ*. The two muscles are separate in all other salamanders (Noble, 1922). I have ascertained that the two are fused in *Hynobius* and in *Onychodactylus*.
6. Larvæ of *Ambystomidæ*, *Salamandridæ*, and *Plethodontidæ* have the first ceratobranchials fused with the second basibranchial (Smith, 1920). This fusion does not occur in larvæ of *Cryptobranchidæ* or of *Hynobiidæ*.

Within the superfamily *Salamandroidea* the *Ambystomidæ* and the *Salamandridæ* are about parallel. The long posterior process of the prevomer distinguishes the *Salamandridæ*, and as the parasphenoid tooth patches of *Plethodontidæ* are the morphological equivalent of this process (Wilder, 1920) it is probable that some primitive Salamandrid (having the two otic elements free) gave rise to the much degenerate *Plethodontidæ*. The

mountain brook habitat of the ancestral Plethodontid (Wilder and Dunn, 1920) accounts perfectly for the retention of the columella through adult life as a working part of the sound-transmitting apparatus.

The *Cryptobranchoidea* contains two families. Of these the *Hynobiidæ* is the more primitive. The *Cryptobranchidæ* differ in lacking the lachrymal bone, in the larval position of the vomerine teeth, and in the much depressed form of the body and head, the last two evidently adaptations for aquatic and bottom-living habits. Besides the characters mentioned in the list as aligning the *Cryptobranchidæ* with the *Hynobiidæ*, several minor points also show this relationship. Both *Ranodon* and *Hynobius* frequently have a lateral fold between the insertions of the legs. This is very prominent in both *Cryptobranchus* and in *Megalobatrachus*, and is not found elsewhere. *Onychodactylus* larvæ have a marked fold on the posterior side of the limbs. This is seen elsewhere only in *Cryptobranchus* and in *Megalobatrachus*.

Inasmuch as the characters differentiating the two genera of *Cryptobranchidæ* have not been clearly understood in the past they are here stated.

Megalobatrachus,	Two persistent branchial arches:
	Frontal not entering naris:
	Branchial clefts closed in adult.
Cryptobranchus,	Three persistent branchial arches:
	Frontal entering naris:
	Branchial clefts open in adult.

In all three of these characters the American genus shows greater adaptation to aquatic life. The European fossils of this family appeal to *Megalobatrachus* in the one skull character which separates the two genera. Neither in *Andrias schuchzeri* nor in *A. tschudii* does the frontal enter the naris.

It is also interesting to note that *Megalobatrachus* shows no "Derotreme" characters whatever, although in the older classifications it was included in the *Derotremata*.

The extreme antiquity of the *Caudata* can be readily seen when an end form, a river adaptation, is found in Oligocene times.

This of course puts the origin of the main stocks back at least to the end of the Mesozoic, a conclusion to which the distribution also forces us.

The primitive characters appear in widely scattered and rather unrelated forms. The free prearticular has already been mentioned. A free lachrymal is found in *Hynobiidæ* and in an Ambystomid, *Rhyacotriton.* A postfronto-squamosal arch is found in one group of the *Salamandridæ.* A T-shaped parasphenoid is found in an Ambystomid (*Dicamptodon*) and in a Salamandrid (*Tylototriton*). Long maxillæ are found in the two forms just mentioned and in another Salamandrid, *Pachytriton.* Posteriorly projecting prevomers are found in *Amphiuma,* in all *Salamandridæ,* in some *Hynobiidæ* (*Hynobius, Pachypalaminus*), and to a less extent in *Dicamptodon.*

All these are theoretically primitive skull characters of amphibians. Their appearance separately in diverse forms is sufficient indication that the three families *Hynobiidæ, Salamandridæ,* and *Ambystomidæ,* while containing all the more primitive forms of the order, stand in no direct genetic relationship to each other, but must be derived from a more or less remote common stock which combined the otic apparatus, lachrymal, and prearticular of *Hynobius* with the long maxilla, T-shaped parasphenoid, and postfronto-squamosal arch of *Tylototriton.*

The evidence of Paleontology, as far as it goes, supports this view. I intend in a later paper to assemble the meager facts regarding fossil salamanders. These facts, it may be here stated, lend no support to the prevalent view that the *Proteida* are an old, a primitive, or an ancestral group.

The following outline classification indicates the size and position of the modern groups. The genera and

species of the *Salamandridæ* are probably not wholly accurate. Future work will perhaps indicate the affinities of *Amphiuma,* the *Proteidæ,* and the *Sirenidæ.*

Of the larger families, the *Hynobiidæ* are entirely Asiatic, the *Salamandridæ* are Eurasiatic with four American species, the *Plethodontidæ* are American with two species in Europe and four in South America, and the *Ambystomidæ* are American with one Asiatic species. As the Northern land masses have been connected with each other during Tertiary times this distribution is not extraordinary, although close resemblance between widely separated species is eloquent testimony as to the antiquity of some of the "modern" forms.

Twenty-two of the recognized genera and 105 of the species are restricted to North America, 13 genera and 56 species are Eurasiatic, while three genera are found both in North America and in some parts of the Old World.

Mutabilia
  Salamandroidea

1. Ambystomidæ ....3 genera, 16 species.
   Dicamptodon 2, *Rhyacotriton* 1, *Ambystoma* 13.
2. Salamandridæ ....7 genera, 37 species.
   Salamandra 5, *Chioglossa* 1, *Tylototriton* 2, *Pachytriton* 1, *Pleurodeles* 3, *Triturus* 24, *Salamandrina* 1.
3. Plethodontidæ ....16 genera, 83 species.
   Desmognathus 7, *Leurognathus* 1, *Plethodon* 11, *Ensatina* 3, *Hemidactylium* 1, *Aneides* 4, *Batrachoseps* 6, *Stereochilus* 1, *Typhlotriton* 1, *Typhlomolge* 1, *Gyrinophilus* 2, *Pseudotriton* 5, *Eurycea* 10, *Hydromantes* 3, *Œdipus* 23, *Œdipina* 4.

  Amphiumoidea (Relationships uncertain, possibly should stand as a family under Salamandroidea)

4. Amphiumidæ ....1 genus, 2 species.
   Amphiuma 2.

Cryptobranchoidea

5. Hynobiidæ ....5 genera, 20 species.
Hynobius 15, *Pachypalaminus* 1, *Onychodactylus* 2, *Ranodon* 1, *Batrachuperus* 1.

6. Cryptobranchidæ ....2 genera, 2 species.
Megalobatrachus 1, *Cryptobranchus* 1.

Proteida (Relations uncertain)

7. Proteidæ ....2 genera, 3 species.
Necturus 2, *Proteus* 1.

Meantes (Relationships uncertain)

8. Sirenidæ ....2 genera, 2 species.
Siren 1, *Pseudobranchus* 1.

Total number of genera 38, of species 165.

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AGENCIES WHICH GOVERN THE DISTRIBUTION OF LIFE

A. BRAZIER HOWELL

Pasadena, Calif.

The problems presented by the distribution of plants and animals is a fertile field for investigation. These problems are essentially ecological in character, for often, perhaps always, the range of a species or genus is dependent upon a number of diverse environmental factors, some of which are readily apparent, while others are obscure; but always they merit careful study.

In investigating and mapping the ranges of living organisms and in following the evolutional tendencies of species in so far as we are able, environment and its influences are of the greatest moment, especially from an ecological standpoint. Botanical subjects may usually be allocated in relation to their surroundings with considerably greater ease than can active forms of life, for the former are acted upon only by the agencies to be found in one spot, while the latter may experience not only all the influences operative over several square miles, more or less, of diversified territory, but, in the case of a migratory bird or mammal, will be subject during a part of the year to environmental factors of which we may know nothing. Whether a species is common or rare in a certain area depends upon its rate of reproduction, which is usually entirely adequate unless new and disturbing influences have been introduced; upon the number of favorable or unfavorable conditions which it encounters, the amount of competition with which it has to contend, and its phylogenetic characters, as to whether it be of a plastic type or one which is senescent and overspecialized: all of which may be summed up in the phrase "adaptability to its habitat."

In any one realm, or larger region of the earth's surface, there are various climatic divisions, the chief of which have been named zones, and these stretch across the continent following isotherms, or mean temperature bands, usually, for our purpose, based upon the average amount of heat present during the three chief reproductive months. Zones are divisible into faunal districts, whose bounds are limited by conditions of humidity, precipitation and a few other causes that may be operative over considerable areas. These are further divisible into associations, an almost limitless number of which may be recognized. Thus, we have littoral, riparian or stream bank, palustral or marshy associations, the latter being capable of still narrower subdivision into tule, arrow-head or salt grass associations, and so on without end.

Associations are sometimes but little considered in parts of the country where climatic conditions are uniform over a wide extent of territory; but in the mountainous parts of the west, where practically every possible local environment from the hottest, most arid deserts, to arctic-alpine conditions may be encountered within a few miles, the importance of their recognition can hardly be overestimated.

In considering the agencies governing the range of a form, the question of temperature is undoubtedly of chief importance as a usual thing, but in some cases physical barriers should be given greater weight, for it need hardly be indicated that it is such directly—and temperature only indirectly if at all—that keep many forms of life from greatly increasing their ranges. In studying such barriers, manner of dispersal may be of much importance. In the case of plants more than of vertebrates (with few exceptions), human agency must now be taken into account, for the activities of man, both intentional and unintentional, are responsible in greater degree for the widespread dissemination of seeds and insects over vast stretches of the earth's surface than

any other cause. Natural manner of dispersal must also be carefully scrutinized as a preliminary step, for what will prove a barrier to the extension of the range of a plant with what I may term unadorned seeds may be inoperative in the case of seeds adapted to dispersal by the wind, and again, those whose covering is fitted for adhesion to the coats of mammals will often be still more widely scattered.

In the case of an animal, the first thing to be considered is the life-type to which it belongs, the chief divisions of which are aquatic, fossorial, terrestrial, arboreal and volant types, which are limited in varying degrees by physical barriers. To an aquatic form, land masses are insuperable obstacles, while to many terrestrial species, especially such as live in very arid regions and are totally independent of water, even a large river may prove a delimiting agent. A strip of rocky country or an extent of arid plain will prevent the spread of such a fossorial mammal as the mole. Arboreal forms are checked by large, treeless areas, and animals which are adapted to a life on the plains will usually shun the forests. Volant types are the most independent of physical barriers of all, and to some even wide stretches of ocean are no obstacle, as in the case of the Pacific Golden Plover (*Charadrius dominicus fulvus*), in its annual migrations between Alaska and the Hawaiian Islands.

While conceding that temperature is the most important factor in the distribution of life, the writer is of the opinion that not enough importance has been credited to other agencies. Dr. C. H. Merriam was, I believe, the first to formulate the theory that the northward range of a species is governed by the mean amount of heat present during the season of reproduction, while the southward range of northern forms is restricted by the mean temperature during the very hottest portion of the year. Isotherms have been determined and our continent plotted and mapped into zones, called Arctic, Hudsonian, Canadian, Transition, Upper Sonoran, Lower

Sonoran and Tropical, some of which are known by other terms in the eastern part of the country. Roughly, the position of an isotherm, as well as the temperature of a region at other times of the year, depends upon latitude, altitude and distance from the sea. Hence it is that the winter temperature of parts of Montana at a considerable altitude and far from the sea reaches a lower figure than has been recorded on the coasts of the Arctic Ocean. The coldest temperature ever known upon the face of the earth—minus 92 degrees F. in the interior of Siberia—is much lower than has ever been found by any of the "farthest north" expeditions.

We may safely infer that the degree of winter cold, below a certain point, is largely immaterial, for it makes no difference to a tree whether the thermometer is ten or sixty degrees below zero, nor to the lesser vegetation and many rodents safely protected by a deep blanket of snow. Even to the few species of birds which habitually spend the winter in high latitudes, very low temperatures are seldom disastrous, but rather is it due, when numbers perish, to a failure of the food supply during sleet storms or long blizzards. Neither birds nor mammals migrate so much because of cold as because their usual foods are not to be obtained in adequate amounts during the winter.

Certain forms of life may have to contend, in relatively low latitudes and altitudes, with conditions which approximate those to be found much farther north. W. T. Shaw has but just brought to our attention the fact that in eastern Washington, where Upper Sonoran conditions are the rule, estivation and hibernation of the Townsend Ground Squirrel (*Citellus townsendi*) are so long continued that this animal enjoys but four months of activity during the year. The squirrels emerge as soon as the first growth starts in the spring, but retire to their burrows for the long sleep when the arid conditions of early summer cause a desiccation of their food supply. To a torpid animal in its nest below ground it

makes no difference whether it is summer or winter above, and so these squirrels seem to lead an existence closely similar to that of their near kin at the Arctic Circle, but with the probable difference that the northern forms experience an actually greater number of hours of daylight throughout the long arctic summer months.

In the plains section of the interior, zonal divisions are acted upon by comparatively few modifying agencies, and their boundaries are rather regular and easily defined, but in parts of the three Pacific Coast states, whose shores are bathed by warm ocean currents, and where the topography is decidedly irregular, the problem of zonal definition is often extremely complicated. In the coast region of northern California, for instance, there is but slight daily and seasonal change of temperature, and a number of Boreal forms are able to occur there because the summers are cool enough for them, while certain Sonoran species are also able to exist because the mean temperature of the breeding season is high enough for their needs. The result is a confusion of zonal indices that is extremely puzzling at first glance.

To these three widely-recognized zonal factors, when operative in certain regions, should undoubtedly be added character of the coastal sea currents—whether warm or cold—and direction of the prevailing winds.

Faunal conditions depend largely upon humidity as well as upon all zonal factors. The chief cause of a humid climate is, of course, ample precipitation, either rather evenly distributed throughout the year, or else supplemented during the drier season by heavy fogs and dense forests to retard evaporation, while a cool climate is often helpful. Precipitation may be largely dependent upon the position of adjacent mountain masses with respect to the prevailing winds, for, as is well known, moisture-laden air is cooled upon contact with an elevated land mass, and precipitation results; but little moisture will then be left in the clouds for rain in the trans-montanic sections. This fact is beautifully

shown by the humid and heavily forested coast and mountain areas of northwestern Washington, in contrast to the bare, arid plains east of the Cascade range.

Associational temperature is induced by many causes, and although limited in extent it profoundly influences local zonal boundaries. Even associational factors other than temperature may raise barriers to distribution that are insurmountable to many organisms.

Insolation, or the relative amounts of sun and shade received by a species in its habitat, is sometimes of paramount importance. This may be influenced by cloudiness, by the amount and density of surrounding vegetation or by the character of the topographical environment. In illustrating this point, we may mention as extremes the bottom of a deep, narrow, forested gulch, and the top of a warm, bare ridge; the face of a steep north slope, and one facing south. A gully on a north slope may be so situated as never to receive the rays of the sun, while at a certain optimum angle one facing towards the south will receive forty per cent. more solar heat than will a level surface. Hence, zonal boundaries upon the two slope aspects will be found to occur at very different altitudes. Soil conditions are of great importance in influencing the temperature immediately above its surface, and its character helps to control both the amount of evaporation and the degree of moisture which it is capable of retaining. A light-colored soil is considerably cooler, other things being equal, than a dark, rocky one, which will absorb and retain more heat. The importance of the chemical composition as well as the mechanical condition, with amount of humus, acid or alkali, in the soil need be no more than mentioned.

The temperature of the soil and the atmosphere above it is often greatly influenced by near-by cold mountain streams, and in places zonal boundaries may be depressed one or two thousand feet in altitude by this agency. Large snowbanks and glaciers have a similar effect, though usually less pronounced or, rather, more

locally restricted. A forest fire or avalanche, by destroying ground shade with the consequent raising of the soil temperature, will usually cause an area to grow up to plants and trees of the zone immediately below, to be gradually restored, in future years, to its original zonal status. Base level has its effect, for the foot of a mountain mass rising from a plain five thousand feet in altitude will have lower zonal tendencies than will the five thousand foot level of a mountain rising from a plain with an elevation of but one thousand feet, because the higher plain accumulates more heat. Similarly, a large mountain mass is less influenced by the conditions which surround it than is an isolated peak. A steep slope will carry a certain zone to a greater height than will a gentle one, because the former will receive, during the day, more of the warm air arising from the lowlands, and the cold air which descends during the night will flow off more rapidly. However, this rule is often nullified by the steep slope being so situated that it receives less sunlight than the more gentle gradient. These points are finely illustrated on most of the mountains of the southwest. Plants and trees of the Transition Zone often flourish on the bottom of a north-facing canyon, while the Sonoran sagebrush extends a couple of thousand feet higher upon the steep slopes with southern exposures.

Protective cover is important to most of the more retiring forms of active life, and to such it is not only necessary as a screen during their daily foragings, but they must have holes into which they may dart at the approach of danger and safe retreats in which to rear their young. To very few vertebrates is the actual character of the soil of great moment, but there are exceptions, as instanced by the large kangaroo rat, *Dipodomys deserti,* the front feet of which are so weak that it seems able to burrow only in deposits of æolian or other loose sand, and it is useless to expect to find this species in hard soil. Needless to say, character of food, both gen-

eral and specific, is a powerful determining factor of distribution, and with this should be classed not only the manner of feeding but the methods employed in securing sustenance. The search for a favorite food item will even, in time, indirectly change a mammal from a terrestrial to an arboreal type, as it evidently has the tree mouse, *Phenacomys longicaudus,* of the coasts of Oregon and northern California, which, so far as known, feeds exclusively upon the needles of coniferous trees.

The question of enemies, it seems to me, should be given much more weight in distributional problems than it usually receives. This factor may be divided into active and passive enemies. By the latter term is meant competitive forms, as the more robust growth that chokes out a tender seedling, or an organism which, being more adaptable to a variety of conditions, forges ahead of less plastic forms whose habits are competitive. It is the opinion of the writer that such competition constitutes the real remorseless struggle for existence which most species are obliged to carry on in order to survive, rather than their efforts to elude their active enemies. Although these passive enemies are not spectacular and are apparent only after scrutiny by an understanding person, they are, nevertheless, always present and operative.

Active enemies may be divided into irritating and exterminating types, and in certain sections the former may constitute a formidable barrier to dispersal. Few of the larger parasites directly cause death, but the presence of great quantities of aphis, scales, ticks or intestinal worms upon their respective hosts may so handicap a species that it is forced to the wall by the competition of more favored forms. Unusual numbers of horse flies in a mountainous section may so harass stock that they utterly refuse to dwell in such regions.

Exterminating agencies may consist of directly predaceous organisms, such as carnivores which consume the flesh of their victims, or rodents whose presence in

great numbers seriously interferes with the propagation of certain plants. The overstocking of a range with cattle or the presence of a vast colony of prairie dogs may actually extirpate certain grasses in those districts, and hordes of some rodents will prevent reforestation in spots because all tree seeds are eaten as fast as produced. Poisonous plants work great havoc among range stock at times, and although the amount of such devastation among wild forms has seldom or never been investigated, it is doubtless an appreciable factor. In some regions, bacteria and disease, including the smaller parasites, play a most important rôle. The tse-tse fly in portions of Africa has rendered it utterly impossible for certain herbivorous mammals to be kept in the infested districts; the *Stegomyia* mosquito that is instrumental in the spread of yellow fever probably caused the Mayan survivors of this dread disease to abandon the ancient civilization of Yucatan, which was at one time so densely populated, and many ailments, comparatively harmless to white men, who have developed a degree of immunity to them, are largely responsible for the decrease in the numbers within recent years of the more savage peoples. From time to time either totally new bacterial diseases appear or else old ones suddenly acquire new virulence, and throughout the ages, such have undoubtedly killed off certain species from faunal divisions; and it is not at all improbable that during the course of bacterial evolution whole genera, or even families, have been exterminated by this agency.

It seems advisable to append to the present paper a chart, or key, to the factors chiefly responsible for the distribution and restriction of the ranges of living forms, but this is submitted with considerable hesitancy. Most of the factors mentioned are so interdependent upon others that it is merely a matter of personal opinion as to which heading they should be placed under. For instance, it is impossible to decide whether the effect of a cold mountain stream should better be listed under zonal

or associational conditions, for it is operative in both connections. It should be understood, therefore, that the arrangement is only tentative, and that the list has been made to conform to the viewpoint of a vertebrate zoologist.

Factors to be Considered in the Dispersal of Life

Life Types:

- Active Forms.
  - Aquatic.
  - Fossorial.
  - Terrestrial.
  - Arboreal.
  - Volant.
- Sedentary Forms.
  - Character of Habitat.
    - Manner of seeding or reproducing.

Direct Physical Barriers:

- Oceans, rivers, etc. (to land forms).
- Land masses, mountains, etc. (to aquatic forms).
  - Forests, plains, deserts, etc.
    - Protective cover.

Regulation by Temperature:

- Zonal

Latitude. Altitude. Proximity to sea. Ocean currents. Prevailing winds.	Mean temperature during reproduction. Mean maximum. Mean minimum. Delimiting temperatures (as frost to tender species).

- Faunal.
  - Humidity.
    - Precipitation.
      - Location of near mountain masses, if any.
      - Location of near bodies of water, if any.
- Associational.
  - Degree of insolation.
  - Effects of fires and avalanches.
  - Presence of cold streams or glaciers.
  - Topographical situation.
    - Slope aspect.
    - Slope angle.
    - Base level.
  - Soil.
    - Chemical and mechanical character.

Color.
Moisture.

Food:

General and specific character.
Feeding habits.

Enemies:

Passive (competitive forms).
Active.
Irritating.
Exterminating.
Directly predaceous.
Poisonous foods.
Bacteria, protozoa, etc.

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THE TAPEWORM INFECTION IN WASHINGTON TROUT AND ITS RELATED BIOLOGICAL PROBLEMS

PROFESSOR NATHAN FASTEN

Oregon Agricultural College, Corvallis, Oregon

In the whole realm of nature man is the only creature whose ailments have seriously occupied the attention of experts. Let a disease break out amongst the human family in some corner of the globe and almost immediately the affliction becomes the target for the trained minds of our ablest pathologists. Not so, however, with the maladies of the lower forms. Man's only interest in them has been one of selfish exploitation, and he has done little to encourage investigations along any other lines except those which bring him immediate monetary returns. It is, therefore, not at all surprising that we possess such meager and fragmentary knowledge concerning disease amongst the lower animals.

It is almost superfluous to say that this attitude must change if we are to intelligently conserve the lower creatures as natural resources. In the last few years we have been hearing a great deal about the conservation of natural resources, and yet very few of us realize the full meaning of conservation. To my mind real conservation implies a thoroughgoing knowledge of the objects to be conserved, coupled with an intelligent application of the factors controlling their preservation. We must possess more knowledge concerning the diseases of the lower animals because it is of prime importance in all conservation programs, in that it may be helpful in preventing great losses of animals which are beneficial to man.

In the state of Washington, as well as in the other states of the Pacific coast, fish afford a natural resource of tremendous importance to the welfare of a large pro-

portion of the citizens, and yet comparatively little is known regarding the diseases which affect these aquatic animals. We become alarmed when the fish begin to die in great numbers, and only then are we in any manner concerned with finding out what ails them.

During the summer of 1919 it was my good fortune to be chosen by the Washington State Fish Commission as a special investigator for the purpose of studying the parasites of the fish in some of the fresh-water lakes and streams of the state of Washington. Prior to undertaking these investigations reports had been coming in to the fish commissioner's office that the fish were dying in the mountain lakes and streams of Kittitas county and, therefore, it seemed advisable to spend most of my time in this region studying the nature and extent of the disease. It is with this epidemic in particular that I wish to deal in the present paper. Incidentally, I desire to point out some of the interesting biological problems with which the question is intimately linked up.

On arriving in Kittitas county the writer found that the people, especially the sportsmen, were very much disturbed about the mortality of their lake trout, for they depended upon these fish to yield them spawn for their county hatcheries. They were particularly distressed about the dying of the trout in Cooper lake, and therefore this lake was the first one which I visited.

Cooper lake is situated in the heart of the Cascade mountains about thirty miles outside of Roslyn. Figures 1–3 show various views of the lake. It is a clear body of water, filled with cut-throat trout. The county game commissioners closed the lake some six years ago in order to obtain a plentiful supply of fish for breeding purposes, and as a result of this the trout have multiplied very rapidly within its waters. For the first few years the results obtained were excellent, but within the last two years the fish commenced to die at an alarming rate, so that all spawning operations had to be abandoned.

An examination of the cut-throat trout of this lake

showed them to be heavily parasitized with larval tapeworms which attack the abdominal cavity. From all appearances these larvæ somewhat resemble those described by Professor Linton in 1889 for the trout of Yellowstone National Park, and, undoubtedly, belong to the genus *Dibothrium* or *Diphyllobothrium,* but are probably of a

Fig. 1. General view of Cooper lake.
Fig. 2. Region of Cooper lake showing racks, a favorite place for the blue herons.
Fig. 3. Shallow portion of Cooper lake near shore affording an ideal nesting place for fish-destroying birds.

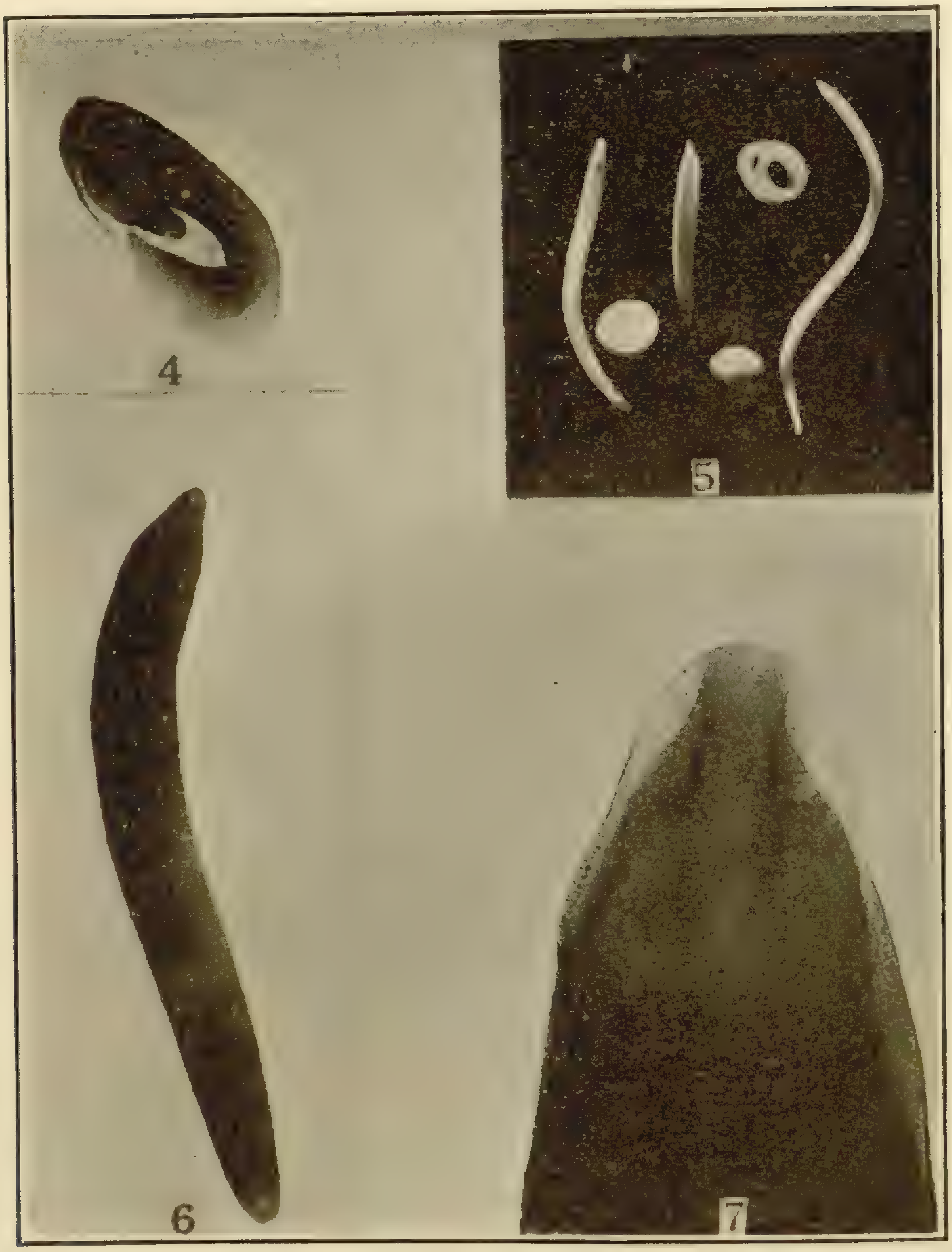

FIG. 4. Tapeworm larva in cyst, × 20.
FIG. 5. Numerous free-boring and encysted tapeworm larvæ, × 3.
FIG. 6. Enlarged photograph of tapeworm larva, × 2[illegible]
FIG. 7. Head end of tapeworm larva, × 65.

different species from *Dibothrium cordiceps* Leidy, the ones discussed by Linton. According to Professor A. R. Cooper, of the University of Illinois College of Medicine, to whom specimens of the tapeworm larvæ were sent for identification, "the placing of these larvæ specifically is a matter of the working out of the life histories of the species in question."

The larval tapeworms under consideration may be en-

cysted (Figs. 4 and 5) along the walls of the digestive tract, particularly on the stomach, or they may be found burrowing freely amongst the visceral structures, or within the surrounding muscular walls. In appearance they are translucent, whitish or yellowish-white organisms which may vary from a few millimeters to about twenty millimeters in length. They are long, slender and worm-like in character (Figs. 5 and 6). At the anterior end is the head (Fig. 7), which possesses two lateral slits. This head end is constantly changing its shape in the living specimens, becoming slender and spear-like at one time and stouter and knob-like at another time. The body proper of the larva may undergo periodic contractions and extensions. Covering its entire outer surface are stiff, bristle-like structures which, at first glance, seem to resemble cilia, but which do not possess any independent motion. Posteriorly the body tapers off into a blunt rounded margin (Fig. 6).

The damage done to the fish by these larval tapeworms is considerable. In the first place, the fish lose their healthy appearance, becoming much thinner and paler in hue. The parasitic larvæ undoubtedly produce injurious toxins which interfere with the proper functions of the host. Then, again, the burrowing habits of these parasites injure the tissues of the fish, causing them to become mushy. And finally, secondary infections of a serious sort may develop within the injured portions. As a result of all this damage great numbers of the fish die.

The life history of these larval tapeworms is extremely interesting. Those who are familiar with tapeworm infection know that ordinarily two organisms are necessary for the completion of the life history. The adult tapeworm lives in one animal called the *primary host*, whereas the larval tapeworm dwells in another animal called the *secondary host*. The primary host becomes parasitized by eating the infected portions of the secondary host.

In the case of the tapeworm under consideration it is quite obvious that the trout acts as the secondary host.

The primary host, however, is not definitely known. Professor Linton found that in the case of the infection of the trout of Yellowstone Park the white pelican acted as the primary host, and, in the light of this finding, it is quite probable that some similar fish-eating bird is the primary host of the larval tapeworm under discussion.

While at Cooper lake a canvass was made of the common fish-eating birds which visit the lake, and it was found that the blue heron is the most frequent visitor. Since no pelicans are known to come to the lake, I rather strongly suspect that the blue heron acts as the primary host for the larval tapeworms of the trout. If this should prove to be the case then the life history, in all probability, would be as follows: The adult *Diphyllobothrium* tapeworm develops in the intestinal tract of the blue heron, and when the segments become mature they are periodically passed out with the fæces. These mature segments contain large numbers of developing embryos and if they are deposited in a stream or lake the embryos are swallowed by the fish, in which they develop into the larval tapeworms already described. When a blue heron captures one of these infected fish, the larvæ attach themselves to the bird's intestinal wall and shortly develop into adults capable of carrying on the life cycle.

My visit to Cooper lake convinced me that it was pure folly to entirely close down a lake for more than a year or two. In the first place, closing down a lake makes for a rapid increase of fish so that the available food supply soon becomes inadequate for maintaining all of them, with the result that a fierce struggle for existence ensues, in which many of the weaker, but nevertheless desirable, fish are killed off. Even those which survive in the struggle appear to be starved. Secondly, when a lake is closed its shores afford an ideal, undisturbed nesting place for such fish-destroying birds as the blue heron, kingfisher and the like. These birds not only destroy large numbers of fish, but they may be the means of disseminating parasitic infections. And lastly, in the light

of the experience in other states, it is a useless waste of money to depend on the fish in a large natural body of water for spawn, because it is very difficult to control the factors which insure success.

Two other mountain lakes were next visited: Lost lake on Roaring creek, near Keechelus, and Fish lake.

Lost lake is stocked with eastern brook and cut-throat trout, with the former predominating in much larger numbers. The lake has been closed for several years and was utilized by the county game commissioners as a place for obtaining eastern brook-trout spawn. From this lake seventy-six brook-trout and two cut-throat trout were examined, and with the exception of two brook-trout all the fish were found to be clean and healthy. The two exceptions mentioned were each parasitized with a single larval tapeworm cyst.

The situation at Lost lake seemed very striking as well as significant, and it suggested the possibility that perhaps the brook-trout are more resistant and immune to the parasitism of the tapeworm larvæ. At any rate, this is worth while testing out much more thoroughly.

One other point which the trip to Lost lake strengthened was in regard to what has already been said concerning the food supply of a closed lake. The fish in this lake, although they were nearly all healthy, were nevertheless very thin. The most prominent parts of them were their heads. In two cases the fish were so hungry that they captured field mice which probably attempted to swim across the lake. These were found partially digested within the stomachs of the fish.

At Fish lake one hundred and nine trout were caught, mainly of the cut-throat species, and a careful examination revealed the fact that they were all healthy and clean. There wasn't a single indication of tapeworm infection. Fish lake was an open body of water and this probably accounts for the healthy state of the fish. When sportsmen can get into a stream they are a source of disturbance to the blue heron and other fish-eating birds, and.

therefore, these birds are prevented from nesting along the shores, thereby protecting the stream from becoming infected with the tapeworm disease.

At the termination of the investigations in Kittitas county, the writer made the following specific recommendations to the county game commissioners:

1. Not to close lakes for more than a short time, say a year or two, and only for the purpose of conserving the fish. When a lake is closed for many years the normal multiplication of fish is such that the food supply within the lake is greatly diminished, resulting in a starvation process. Furthermore, unless adequate watch is maintained, the heron and other fish-destroying birds will live along the shores of these closed lakes and serve as a constant source of infection for the fish.
2. Not to depend on the closed lakes for spawn, but instead to develop a hatchery or a series of hatcheries with numerous outdoor ponds where they can place many of the healthy trout from Lost and Fish lakes, which will give them a constant supply of healthy spawn. They will not only save money by such a project, but their efforts will not be wasted.

After the completion of the above studies the writer examined fish from various places in King county, in which he has found the same larval tapeworm infection. Numerous cut-throat trout of Klause lake near Snoqualmie falls were examined and found to be heavily parasitized. Also, the silver salmon and the so-called red fish or silver trout (which are nothing more than land-locked sockeye salmon) were found to be heavily infected with the same parasites. The striking thing about the parasitism of these last-named fish was that they were more heavily parasitized than any of the fish previously examined in which the tapeworm larvae were found to dwell.

The observations recorded in the present paper make it obvious that a good many of our fish and game cultural practices are utterly wasted because we are ignorant of those factors which ought to insure success. What is urgently needed in the state of Washington as well as in the neighboring states of the Northwest is a series of "Biological Surveys" for the purpose of studying and mapping out the various ecological factors of the regions in which fish or game are to be planted. We ought to know a good deal about such factors as available food supply, oxygen content, temperature variations, predatory and parasitic organisms, etc., of a place before any kind of animals or plants are introduced into it. Knowing these conditions we can then intelligently fit each organism into that particular environment where it will thrive best. But without this knowledge we are simply groping in the dark and are powerless to do any real good.

MIGRATIONS AND AFFINITIES OF THE FOSSIL PROBOSCIDEANS OF EURASIA, NORTH AND SOUTH AMERICA, AND AFRICA

(Sixth contribution on the evolution of the Proboscidea)

DR. HENRY F. OSBORN

American Museum of Natural History

Dr. Hikoshichiro Matsumoto, of the Tôhoku Imperial University, Sendai, Japan, has recently been studying the Fayûm collections of primitive proboscideans and hyracoids in The American Museum of Natural History, followed by a visit to the British Museum where he has been making comparisons with the types of these mammals, described by Dr. C. W. Andrews in his series of papers beginning in 1901. In 1918 Doctor Matsumoto[1] published a series of five papers on the elephants, turtles, sirenians, cervids, and bisons of Japan compared with those of India. He pointed out that the Japanese archipelago was an integral part of the continent from the beginning of the Miocene to the middle of the Pleistocene, and that the period of separation seems to have dated from the recent Pleistocene. Consequently its relations with the animal life of southern China and with the more distant peninsula of India are very close.

The ancient Japanese proboscideans are chiefly of three kinds, of which the most numerous are the forest-living stegodonts, closely related in their specific phases to the stegodonts of China, such as the species *Stegodon sinensis*. There also occurs in the early Pleistocene the

[1] 1. "On a New Archetypal Fossil Elephant from Mt. Tomuro, Kaga." 2. "On a New Fossil Trionyx from Hokkaido." 3. "A Contribution to the Morphology, Palæobiology and Systematic of *Desmostylus*." 4. "On a New Archetypal Fossil Cervid from the Prov. of Mino." 5. "On Some Fossil Bisontines of Eastern Asia." *Sci. Rep. Tôhoku Imp. Univ.*, Sec. Ser. (Geology), Vol. III, No. 2.

great *Loxodon antiquus namadicus,* the straight-tusked elephant, which ranged all over southern Eurasia and probably arose originally in the African continent.

In the early formations, such as the Middle Pliocene of Tomuro, Kaga, we meet the *Elephas auroræ,* regarded by the author as an intermediate type between the stegodonts of the Upper Pliocene of India and *Elephas planifrons,* which in turn is related to the true mammoths (*Elephas primigenius*) and wandered all over southern Europe in Upper Pliocene time, namely, Bessarabia, Austria, and southern France. In still earlier deposits, such as the Upper Miocene of Kuji, occurs a mammal which the author refers to *Stegodon latidens,* an ancestral stegodont of Burma, India. In the Lower Miocene of the Province of Mino occurs a form very similar to the *Trilophodon angustidens* of the Middle Miocene of France, ancestral to all the long-jawed proboscideans.

The *Stegodon* itself is peculiar to India, China, Japan, and the larger islands of the Malayan archipelago, such as Sumatra, Java, and Borneo. The author notes that there is a marked difference between the sexes, so that the stegodonts of each geologic period seem to have received two specific names, one applied to the female, the other to the male form. Among these couples are *S. Cliftii-bombifrons,* dating from the Upper Pliocene and from the Lower Pliocene of India; *S. ganesa-insignis,* dating from the Upper Pliocene and from the Postpliocene of the same area; *S. sinensis-orientalis,* dating from the same strata of China and Japan; *S. airawana-trigonocephalus* from the Postpliocene of Java. This sex dimorphism is very marked, especially in the great disparity of size of the upper tusks, which are much smaller and more slender in females than in males. This tusk structure in turn affects the entire form of the head.

The *Bison occidentalis* of Japan seems to spring from the *B. sivalensis* of the Upper Pliocene of India. It is similar in fact to the bison found in the ancient Pleistocene of Kansas, in the basin of the Ohio River, in Alaska,

and in the region of the Yenisei River in Siberia. According to the author, in the Transbaikal region the same species occurs in association with the giant woolly rhinoceros (*Diceros antiquitatis*), with the hairy mammoth (*Elephas primigenius*), and with the heavy-horned bison (*Bison crassicornis*).

Quite a different order of distribution has the remarkable *Desmostylus,* a sirenian or sea cow peculiar to the coasts of the Pacific Ocean, first described from the California coast many years ago by Professor Marsh and more recently recorded from Japan. The Japanese species is much more specialized and of larger size than the forms occurring on the Oregon and California coasts, which points to a general migration from east to west, that is, from the Orient to the Pacific coast of North America.

From this series of papers we are able to draw up the following table showing the principal distribution of the species of mammals in the descending order of the deposits in Japan:

Postpliocene of Shôzu-shima (Sanuki): *Stegodon sinensis, S. orientalis, Loxodon antiquus namadicus, Bison occidentalis, Cervus* (*Sika*) cf. *nippon.*

Upper Pliocene of Ikadachi-mura (Omi): *Stegodon sinensis, S. orientalis, Buffelus* sp.

Middle Pliocene of Tomuro (Kaga): *Elephas auroræ.*

Upper Miocene of Kuji (Hitachi): *Stegodon* cf. *latidens.*

Middle Miocene of the Provinces of Teshio, etc.: *Desmostylus japonicus.*

Lower Miocene of the Province of Mino: *Trilophodon* cf. *angustidens, Teleoceras* sp., *Amphitragulus minoënsis.*

The present researches of Doctor Matsumoto on the rich Fayûm collections of the American and British Museums have enabled him to draw an important distinction in northern Africa between the true forest-living mastodons, which appear to be directly descended

from the genus *Palæomastodon* of the Fayûm, and the long-jawed mastodons, which appear to be directly descended from *Phiomia* of the Fayûm. This interesting discovery, which was partly anticipated in Doctor Andrews's own papers, enables us to trace the American mastodon far back into Upper Eocene times of northern Egypt.

In this connection may be mentioned also a series of five papers[2] by the present reviewer on the "Evolution, Phylogeny, and Classification of the Proboscidea" which have appeared successively since 1918. The writer is attempting to give an iconographic revision of the entire group of proboscideans, including the progenitors of Africa and Eurasia and the highly developed descendants of North and South America, which together make up the most remarkable family history of which we have record.

In 1900 Osborn predicted that the source of the mammalian order of the Proboscidea would probably be discovered in Africa. In 1901 Beadnell and Andrews revealed, through the Geological Survey of Egypt, the rich fauna of the Fayûm, southwest of Cairo, in which were found the remains of three proboscidean genera, named by Andrews *Mœritherium, Palæomastodon, Phiomia,* and confirmed by subsequent exploration and research to be the oldest proboscideans thus far known. Animals similar to *Mœritherium* and *Phiomia* have since been reported by Pilgrim in southern Asia. These animals are

[2] The first paper in this series is entitled "A Long-jawed Mastodon Skeleton from South Dakota and Phylogeny of the Proboscidea," *Bull. Geol. Soc. Amer.*, XXIX, March, 1918; the second paper, "Evolution, Phylogeny, and Classification of the Proboscidea," *Amer. Mus. Novitates* No. 1, January 31, 1921 (Osborn, 1921. 515); the third paper. "First Appearance of the True Mastodon in America," *Amer. Mus. Novitates* No. 10, June 15, 1921; the fourth paper appears in the *Bulletin* of the Geological Society of America, under the title "Evolution, Phylogeny, and Classification of the Mastodontoidea"; the fifth paper, "Adaptive Radiation and Classification of the Proboscidea," was read before the National Academy of Sciences, April 26, 1921. The present is the sixth paper. The Iconographic Type Revision will form one of the Memoirs of the American Museum of Natural History.

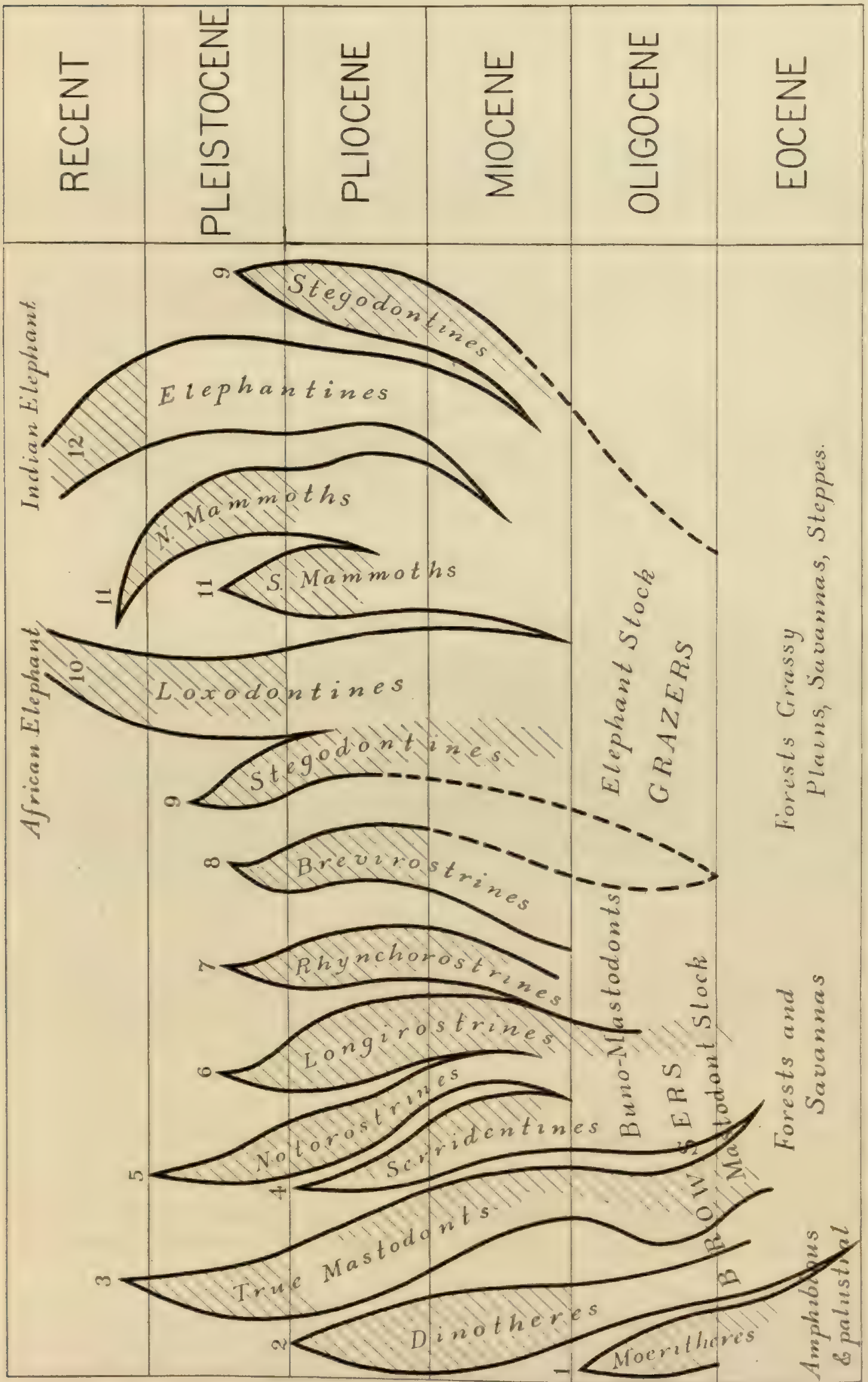

FIG. 1. Diagram showing the present theory as to the adaptive radiation of the Proboscidea. June, 1921.

now found to belong respectively to three distinct lines of the Proboscidea, namely, the mœritheres, the true mastodonts, the long-jawed bunomastodonts, as indicated in black on the accompanying diagram. They point, however, to a long antecedent origin and radiation. This is part of the evidence for an ancient adaptive radiation process by which it now appears that the proboscideans, like other hoofed mammals, were broken up into several great primary stocks way back in Eocene times, namely:

An *amphibious* stock, adapted to rivers and swamps, of limited migration (= *Mœritherium, Dinotherium*).
A *mastodont* stock, adapted to forests and savannas, of wide migration (= mastodonts, trilophodonts).
A *stegodon-elephant* stock, adapted to southern forests, to grassy plains, to savannas and steppes, of wide migration (= *Stegodon, Loxodon, Elephas*).

These primary stocks gave off from two to six branches each, so that the Proboscidea as a whole are not two branched (*i.e.,* mastodonts and elephants), as formerly supposed, but many branched or *polyphyletic.* The forest and savanna browsers and the grazers of the plains and steppes were the long distance travelers and from an African or Asiatic center in Eocene times they reached in the Middle and Upper Miocene all the continents of the world except Australia, while the amphibious forms remained in Africa and southern Eurasia. Certain of these branches, like the true mastodons, are of very great geologic antiquity. Intelligent, independent, well defended, resourceful, adaptive, we find eleven very distinct branches of proboscideans persisting into Upper Pliocene times, five of the least hardy of which became extinct during the colder conditions of the Lower Pleistocene. The known lines of evolution are shaded on the accompanying diagram; the unknown are left in white. The theoretic adaptive radiation may be expressed in a formal classification as follows:

Amphibious and swamp-living stock

I. MŒRITHERIOIDEA (Mœritheres)

1. Mœritheriini,[3] amphibious or swamp-living forms known in the Upper Oligocene of Africa.

II. DINOTHERIOIDEA (Dinotheres)

2. Dinotheriini,[4] large amphibious forms frequenting the

4 *Ibid.*

rivers of southern Eurasia throughout the Miocene to the close of the Pliocene.

Forest and savanna grazers

III. MASTODONTOIDEA (Mastodonts and Bunomastodonts)

Mastodontidæ or "true mastodonts," including the subfamilies

3. Mastodontinæ, springing from *Palæomastodon* of the Oligocene of North Africa, and terminating with *Mastodon americanus* of the Pleistocene forests of North America; grinders lophodont, lacking trefoils.

4. Serridentinæ,[5] first known in the Middle Miocene of France and Switzerland, spreading over into India and North America; lacking the trefoils.

Bunomastodontidæ, the bunomastodonts, springing from forms similar to the *Phiomia* of North Africa and separating into four main divisions:

6. Longirostrinæ, typical long-jawed bunomastodonts arising in North Africa (*Phiomia*), spreading all over southern Europe, Asia, and North America.

5. Notorostrinæ, a special branch entering the Andean region of South America and spreading over the South American continent, distinguished by the loss of the lower tusks and the abbreviation of the jaw.

7. Rhynchorostrinæ, beaked bunomastodonts, known only in the southern United States and northern Mexico, with powerful downturned upper and lower tusks.

8. Brevirostrinæ, short-jawed bunomastodonts, which imitate both the true mastodonts and the elephants in the abbreviation of the lower jaw and the early loss of the inferior tusks.

3 Herluf Winge, 1906, p. 172.

5 It is a question whether this subfamily is nearest the Mastodontidæ, with which its members are generally placed by European paleontologists.

These animals wandered all over Europe, Asia, and western North America.

IV. ELEPHANTOIDEA (the Elephant stock)

9. Stegodontinæ, the original members of which were doubtless ancestral to all the higher elephants, persist as an independent branch into the Lower Pleistocene of eastern Asia.
10. Loxodontinæ, embracing the great African division of the elephants beginning with varieties of the *Loxodon antiquus* of the Upper Pliocene, which wandered all over southern Eurasia and radiated widely over Africa.
11. Mammontinæ, including (*a*) the Southern Mammoths (*Elephas planifrons* of India and *E. meridionalis* of Europe), from which is derived *E. imperator* of North America, and (*b*) the Northern Mammoths, which probably include *E. columbi* and the widespread *E. primigenius* of the northern steppes; (?) tetradactyl pes.
12. Elephantinæ, the true elephants (*E. indicus* of India), which do not appear until the Upper Pleistocene; pentadactyl pes.

This twelve-fold branching of the proboscideans is similar to the adaptive radiation which the author has traced in the evolution of the horses, of the rhinoceroses, and of the titanotheres, carrying the fundamental lines of separation back to the Middle Miocene as the most recent date, and to the Middle or Lower Eocene as the most remote date. It will be observed from the diagram (Fig. 1) that the shaded areas represent those proboscidean phyla of which remains have been discovered. The large unshaded area includes the entire Oligocene, Miocene, and Lower and Middle Pliocene history of the Elephantidæ which is still unknown but which is likely to be revealed at any time by discoveries both in Africa and in central Asia. A very striking fact is that the early member of the Elephantoidæ, the *Elephas planifrons* of the Upper Pliocene of India and the apparent ancestor of the mammoths, is now antedated in geologic time and in its transitional structure by the *Elephas auroræ* (*i.e.*, of the rising sun region) of Japan.

BOOKS AND LITERATURE

The Conservation of the Wild Life of Canada. By Dr. C. Gordon Hewitt, late Dominion Entomologist and Consulting Zoologist. New York: Charles Scribner's Sons, 344 pp., illustrated.

This book was in manuscript before the untimely death of Doctor Hewitt, February, 1920, and has been prepared for publication by his wife. Mrs. Hewitt has also written a beautiful preface which can perhaps be fully appreciated only by those who had the rare good fortune to count Hewitt as a personal friend.

To get the proper perspective on this book, one should know that Doctor Hewitt was a zoologist of broad training. Previous to coming to Canada he had worked not only on insects but also on several problems on birds and their control of insect pests. The record of his work as Dominion Entomologist from 1909 until his death is a brilliant one. Throughout this period he was frequently consulted regarding various zoological problems which came before the Advisory Board on Wild Life Protection and in 1916 he was appointed Dominion Consulting Zoologist which broadened his official interest. The work recorded in the book under discussion was done chiefly during the last four years of his life. For so busy a man to undertake a task of this size and to cover the field so well in so short a time is an enviable accomplishment.

The reading of this book is like a trip to the North Woods, but with a scientist as companion rather than a record-breaking hunter of big game. Although the title might properly include fur-bearing animals and other natural groups, the discussion is chiefly limited to the larger wild mammals and birds of Canada. The information regarding the present distribution and abundance of the several species is accumulated from many sources and constitutes a valuable inventory of the remaining but diminishing resources of the Dominion. As might be expected of one who understands the dangers of promiscuous and ignorant hunting and who appreciates wild life, the dangers and economic loss of unrestricted shooting are constantly set forth, and the results of inadequately controlled slaughter in the United States

are used as an ignoble example. It is not too late in Canada to profit by mistakes south of the boundary, and Doctor Hewitt's book should serve as a timely warning.

Considerable progress has already been made in establishing government and private reserves in Canada and the record of this movement as given in this book is one of the most valuable features of the work. The author took a lively interest in this movement, and in efforts to conserve the wild life of the Dominion he did everything possible, from revising the game laws of the Northwest Territories to the instruction of Boy Scouts in bird protection. Previous to his work the game laws of the Northwest Territories had not been revised for many years, and he succeeded in the difficult task of getting through a revision that is a great improvement over the former regulations. His successful effort to bring about the Migratory Birds Treaty between the United States and Canada was an accomplishment of high order. There were, of course, other earnest men on both sides of the boundary who assisted in this work, but to the author of this book fell some of the most aggravating and ability-testing tasks. If the full history of this effort is sometime written, Doctor Hewitt's part will appear as a large one.

The discussion of the periodic fluctuations of Canadian fur-bearing animals in Chapter IX is perhaps the best example of scientific method in the book. These fluctuations have long attracted the attention of scientific and commercial men and they are here discussed from abundant data and from a biological point of view.

This posthumous book is an additional monument to the scientific skill and personal abilities of the author. It should serve as a valuable warning to Canadians and will be of value to readers everywhere in giving a summary of the resources of the Dominion in one of its most interesting and economically valuable assets. Because of the wide interest in big game it should attract temporarily or permanently to Canada those who retain a wholesome love for the outdoors.

E. F. Phillips

SHORTER ARTICLES AND DISCUSSION

THE PROBABILITY ESTABLISHED BY A CULTURE OF GIVEN SIZE THAT A MATING IS CAPABLE OF PRODUCING ONLY DOMINANT INDIVIDUALS

To distinguish individuals heterozygous from those homozygous for a given dominant factor is a matter of mere inspection when the simplex condition is somatically distinct from the duplex condition, as is the case with the mottling factor in the Adzuki Bean.[1] Generally, however, the degree of dominance is such that a breeding test must be resorted to in order to distinguish these two types. A homozygous dominant will breed true for the character whether selfed or back-crossed to the recessive, whereas a heterozygous individual will give 3 : 1 and 1 : 1 ratios respectively when similarly treated. The common breeding practice is to consider the parent homozygous when, if selfed or back-crossed, it fails to produce any recessive individuals in a reasonably large number of offspring.

Just what is to be considered an adequately large number of offspring has in the past been determined by the personal judgment of the individual investigator, and the difficulty of obtaining offspring in large numbers. There has been no general agreement based on mathematical considerations, probably because large numbers of offspring have not been found necessary in order to distinguish a homozygous dominant from a heterozygous parent producing such ratios as 3 : 1 and 1 : 1. The need of a statistical criterion of what is an adequately large number of offspring was realized when it became necessary in tetraploid races of the Jimson Weed (*Datura Stramonium*) to distinguish between matings which should produce only dominant purple offspring and those which should produce a 35 : 1 ratio of purples to whites. In distributions which are so asymmetrical as those given by sampling from the 35 : 1 ratio, we are hardly justified in using the ordinary theory of probable errors. Special tables have, therefore, been computed for use in work under way at the Station for Experimental Evolution. Since other investigators will probably meet with the need for similar criteria, it seems worth while to give tables showing the number of offspring

[1] *Jour. Hered.*, 8, 125–131, Fig. 10, 1917.

which should be considered in order to distinguish matings which should give all dominant individuals from those which may produce recessives.

The theory is of course quite simple. It is assumed that the expected ratio of dominant to recessive is known, and is $p : q$, where $p + q = 1$. The distribution of the chances of obtaining dominant and recessive individuals in the frequencies $n : 0$, $(n-1) : 1$, $(n-2) : 2$, etc., when n individuals are grown is $(p + q)^n$. To ascertain the probability of securing all dominant individuals in a culture which should show a definite ratio of dominant to recessive offspring we have merely to table p^n against n. If this value is very small, it is reasonable to assume that in practice a culture of n individuals all of the dominant type represents a parent or parents capable of producing only offspring of the dominant type. Thus, for example, if seeds which should produce dominant and recessive individuals in a 5 : 1 ratio were sown, a culture of 35 all dominant individuals should be obtained only 17 times in 10,000. Hence, if a sowing is made to distinguish between a mating capable of producing only dominants and one which should give recessives in a 5 : 1 ratio, and there results a culture of 35 individuals all of the dominant type, it is altogether reasonable to assume that the mating in question is incapable of producing recessives.

Tables have been formed to include the 3 : 1 and 1 : 1 ratios familiar in ordinary disomic inheritance, the 2 : 1 and 8 : 1 ratios found in trisomic inheritance in the mutant Poinsettia, and the 5 : 1, 11 : 1, and 35 : 1 ratios found in tetraploids in *Datura*. Some of these ratios are suggested by published data on *Œnothera Lamarckiana* and *Primula sinensis*, and will probably be found ultimately by those studying other forms.

The tables enable one to decide how large a culture is necessary on a probability basis. If it is felt that only 1 chance in 1,000 of the mating being capable of producing a recessive is sufficient evidence that the culture represents only dominants, then, to distinguish a mating which can produce only dominants from one which should give a 1 : 1 ratio, a culture of at least 10 individuals is necessary. If the 3 : 1 ratio is the one in question, then 24 individuals are necessary; while if a 35 : 1 ratio is considered, 244 individuals are required. In other words, cultures of 10, 24 and 244 individuals are of equal value in distinguishing matings which should produce only dominants from those which

should give, respectively, 1 : 1, 3 : 1, and 35 : 1 ratios of dominants to recessives.

A. F. BLAKESLEE,
JOHN BELLING,
J. ARTHUR HARRIS.

TABLE I

VALUES OF p^n FOR 1 : 1, 2 : 1, 3 : 1, AND 5 : 1 RATIOS

N	1 : 1	2 : 1	3 : 1	5 : 1	N	3 : 1	5 : 1
1....	.5000	.6667	.7500	.8333	19	.0042	.0313
2....	.2500	.4444	.5625	.6944	20	.0032	.0261
3....	.1250	.2963	.4219	.5787	21	.0024	.0217
4....	.0625	.1975	.3164	.4823	22	.0018	.0181
5....	.0313	.1317	.2373	.4019	23	.0013	.0151
6....	.0156	.0878	.1780	.3349	24	.0010	.0126
7....	.0078	.0585	.1335	.2791	25	—	.0105
8....	.0039	.0390	.1001	.2326	26	—	.0087
9....	.0020	.0260	.0751	.1938	27	—	.0073
10....	.0010	.0173	.0563	.1615	28	—	.0061
11....	—	.0116	.0422	.1346	29	—	.0051
12....	—	.0077	.0317	.1122	30	—	.0042
13....	—	.0051	.0238	.0935	31	—	.0035
14....	—	.0034	.0178	.0779	32	—	.0029
15....	—	.0023	.0134	.0649	33	—	.0024
16....	—	.0015	.0100	.0541	34	—	.0020
17....	—	.0010	.0075	.0451	35	—	.0017
18....	—	—	.0056	.0376	36	—	.0014

TABLE II

VALUES OF p^n FOR 8 : 1 RATIO

N	0	1	2	3	4	5	6	7	8	9
1......	.3079	.2737	.2433	.2163	.1922	.1709	.1519	.1350	.1200	.1067
2......	.0948	.0843	.0749	.0666	.0592	.0526	.0468	.0416	.0370	.0329
3......	.0292	.0260	.0231	.0205	.0182	.0162	.0144	.0128	.0114	.0101
4......	.0090	.0080	.0071	.0063	.0056	.0050	.0044	.0039	.0035	.0031
5......	.0028	.0025	.0022	.0019	.0017	.0015	.0014	.0012	.0011	.0010

TABLE III

VALUES OF p^n FOR 11 : 1 RATIO

N	0	1	2	3	4	5	6	7	8	9
1......	.4189	.3840	.3520	.3227	.2958	.2711	.2485	.2278	.2088	.1914
2......	.1755	.1609	.1475	.1352	.1239	.1136	.1041	.0954	.0875	.0802
3......	.0735	.0674	.0618	.0566	.0519	.0476	.0436	.0400	.0366	.0336
4......	.0308	.0282	.0259	.0237	.0217	.0199	.0183	.0167	.0154	.0141
5......	.0129	.0118	.0108	.0099	.0091	.0083	.0077	.0070	.0064	.0059
6......	.0054	.0050	.0045	.0042	.0038	.0035	.0032	.0029	.0027	.0025
7......	.0023	.0021	.0019	.0017	.0016	.0015	.0013	.0012	.0011	.0010

TABLE IV

VALUES OF p^n FOR 35 : 1 RATIO

N	0	1	2	3	4	5	6	7	8	9
3.....	.4295	.4176	.4060	.3947	.3837	.3731	.3627	.3526	.3428	.3333
4.....	.3241	.3151	.3063	.2978	.2895	.2815	.2737	.2661	.2587	.2515
5.....	.2445	.2377	.2311	.2247	.2184	.2124	.2065	.2007	.1952	.1897
6.....	.1844	.1793	.1744	.1695	.1648	.1602	.1558	.1515	.1473	.1432
7.....	.1392	.1353	.1316	.1279	.1244	.1209	.1175	.1143	.1111	.1080
8.....	.1050	.1021	.0993	.0965	.0938	.0912	.0887	.0862	.0838	.0815
9.....	.0792	.0770	.0749	.0728	.0708	.0688	.0669	.0651	.0632	.0615
10.....	.0598	.0581	.0565	.0549	.0534	.0519	.0505	.0491	.0477	.0464
11.....	.0451	.0439	.0426	.0414	.0403	.0392	.0381	.0370	.0360	.0350
12.....	.0340	.0331	.0322	.0313	.0304	.0296	.0287	.0279	.0272	.0264
13.....	.0257	.0250	.0243	.0236	.0229	.0223	.0217	.0211	.0205	.0199
14.....	.0194	.0188	.0183	.0178	.0173	.0168	.0164	.0159	.0155	.0150
15.....	.0146	.0142	.0138	.0134	.0131	.0127	.0123	.0120	.0117	.0113
16.....	.0110	.0107	.0104	.0101	.0098	.0096	.0093	.0091	.0088	.0086
17.....	.0083	.0081	.0079	.0076	.0074	.0072	.0070	.0068	.0066	.0065
18.....	.0063	.0061	.0059	.0058	.0056	.0055	.0053	.0052	.0050	.0049
19.....	.0047	.0046	.0045	.0044	.0042	.0041	.0040	.0039	.0038	.0037
20.....	.0036	.0035	.0034	.0033	.0032	.0031	.0030	.0029	.0029	.0028
21.....	.0027	.0026	.0025	.0025	.0024	.0023	.0023	.0022	.0021	.0021
22.....	.0020	.0020	.0019	.0019	.0018	.0018	.0017	.0017	.0016	.0016
23.....	.0015	.0015	.0015	.0014	.0014	.0013	.0013	.0013	.0012	.0012
24.....	.0012	.0011	.0011	.0011	.0010	.0010	.0010	.0010	.0009	.0009

LINKAGE BETWEEN BRACHYSM AND ADHERENCE IN MAIZE

ADHERENCE first appeared in the second generation of a brachytic x Boone Co. White hybrid and seemed to be linked closely with normal stature.[1] Subsequent progenies indicated that there was no very close linkage between these characters and possibly none at all.[2] The relationship of these two interesting characters has been tested now in more detail and it seems certain that their genes are located on the same chromosome.

A cross was made between a non-adherent brachytic plant and an adherent plant of normal stature, both plants being segregates in the F_2 of the brachytic-Boone hybrid. The first generation segregated with respect to the brachytic culms, approximately half the plants being of normal stature, but none exhibited a tendency toward adherence. From the behavior of the F_1 plants it is apparent that the adherent parent of the cross was heterozygous with respect to the brachytic character.

[1] Kempton, J. H., "A Brachytic Variation in Maize," U. S. Dept. of Agri. *Bull.* 925, Feb., 1921.

[2] Kempton, J. H., "Heritable Characters in Maize V. Adherence," *Journal of Heredity*, Vol. XI, No. 7, Sept.–Oct., 1920.

Three F_1 plants of normal stature were self-pollinated and three were back-crossed on the double recessive (adherent-brachytic). The six ears were planted separately at Arlington, Virginia, but the resulting F_2 populations were not as large as could be desired.

The combined self-pollinated progenies gave the following distribution:

No. Plants				Per Cent.			
Nor.	Br.	Ad.	Br.-Ad.	Br.	Ad.	Crossover	Q.
217	91	85	4	23.9	22.4	22.2 ± 3.4	.798 ± .06

and the plants of the combined back-crossed progenies are distributed as follows:

Nor.	Br.	Ad.	Br.-Ad.	Br.	Ad.	Crossover
86	188	178	71	49.5	47.6	30.0 ± 1.35

These distributions clearly indicate that crossing over between these two factors occurred in from 20 to 30 per cent. of the gametes.

Additional evidence of linkage between these characters is afforded by the second generation of a cross between an adherent plant of normal stature and a ramose-brachytic plant. The F_1 of this cross was normal with respect to all three characters, and they all reappeared in the progenies of the second generation. Five F_1 plants were self-pollinated and the resulting ears planted separately. Unfortunately in most of the F_2 progenies there is a deficiency of adherent plants and for the combined progenies the departure below the expected 25 per cent. is 7.8 ± .87, a deviation too large to be ascribed to chance. Whether this deficiency represents seedling mortality is not known, but at the time the plants were classified many of the progenies contained late plants strikingly smaller and weaker than their mature sisters. Some of these plants consisted of a small cluster of grasslike leaves with inflorescences hardly developed beyond the embryonic stage. Such plants could not be classified with respect to adherence, though in many cases it was possible to determine satisfactorily whether they were ramose or brachytic. With respect to these last two characters

the small late plants approximated the familiar 9–3–3–1 grouping. If the assumption is made that all these late plants were adherent, the percentage of adherent plants in most of the progenies would then approximate the expected. For the present analysis of the relationship of brachytic and adherent, the low percentage of adherent plants is not important, since the percentage of crossovers can be determined from the ratio of normal to brachytic plants or by the use of Yule's Coefficient of Association.[3]

Combining the five progenies the distribution of plants is as follows:

NUMBER OF PLANTS

Nor.	Ad.	Ra.	Br.	Ad.-Ra.	Ad.-Br.	Ra.-Br.	Ad.-Ra.-Br.	Small Plants
361	135	117	193	18	3	57	2	61

PER CENT.

Ad.	Ad. and Small Plants	Ra.	Br.
17.8 ± .87	23.2 ± .92	21.9 ± .94	27.9 ± 1.0

PER CENT. OF CROSSOVERS

Ad.-Ra.		Ad.-Br.		Ra.-Br.	
Q.	%	Q.	%	Q.	%
37 ± .06	38.5 ± 1.8	.886 ± .03	16.8 ± 1.9	.05 ± .06	49.9 ± 0.4

It is seen that the progenies of this hybrid indicate about 17 per cent. of crossing over while the three self-pollinated progenies of the other hybrid, involving brachytic and adherent, indicate 22 per cent. and the back crosses 30 per cent. It seems inadvisable to combine the self-pollinated progenies from the two hybrids to arrive at a single figure for the percentage of crossovers since the degree of crossing over between two factors often varies greatly in different progenies. It seems certain from these two hybrids that these two characters are located in the same chromosome separated by a distance varying from 18 to 30 units, thus making a linkage series of brachytic, adherent and pericarp color.

[3] Yule, G. Udney, "On the Association of Attributes in Statistics," *Phil. Trans. Roy. Soc.*, London, S. A., Vol. 94, pp. 257–319, 1900.

The progenies of the brachytic-adherent-ramose hybrid furnish evidence that the ramose character may belong to the same linkage series, though the linkage is rather loose.

Although the tassels of ramose plants are much larger than those of normal plants and it seemed not unreasonable to expect adherent-ramose tassels to present a large thickened mass, nothing of the sort was found and the ramose-adherent plants could be separated from the normal-adherent plants only by examining the ears.

White and colored seeds were planted separately, but the percentage of the three characters are essentially alike, as is shown by the following figures indicating that all three are independent of one of the aleurone factors:

	% Adherent	% Ramose	% Brachytic
White seeds planted	16.4 ± 1.65	24.3 ± 1.92	31.5 ± 2.04
Colored seeds planted	18.3 ± 1.00	21.1 ± 1.07	27.9 ± 1.17
Difference	1.9 ± 1.93	3.2 ± 2.2	3.6 ± 2.34

J. H. KEMPTON

BUREAU OF PLANT INDUSTRY,
U. S. DEPARTMENT OF AGRICULTURE

A GENE FOR THE EXTENSION OF BLACK PIGMENT IN DOMESTIC FOWLS [1]

THE results of recent experiments on the inheritance of plumage colors in fowls indicate that varieties in which black pigment extends to all or nearly all of the plumage (*e.g.*, self black) differ by one dominant autosomal gene from varieties in which black pigment is restricted to the hackle, flight and tail feathers (*e.g.*, Columbian and buff varieties). This gene has been called "extension of melanic pigment" and has been assigned the symbol E^m.

The evidence is derived from reciprocal crosses between Black Orpington and Columbian pattern (Light Brahma) fowls. Whichever way the cross is made the F_1 chicks are all black in the down. As adults, the males from the reciprocal crosses are alike. They are black with white-bordered hackles and saddle feathers; white-bordered and splashed or stippled wing coverts and narrow white borders on the upper breast feathers. They resemble fairly typical Dark Brahma or Duckwing males.

[1] Contributions in Poultry Genetics, Storrs Agr. Experiment Station.

The females from the reciprocal crosses are unlike in adult plumage. From the cross of Black male by Columbian female, the daughters are self black. From the cross of Columbian male by Black female, the daughters are black with white borders on the feathers of head, hackle, and upper breast. They resemble the pattern known as Birchen.

When backcrossed with Columbian females, the F_1 males have produced black, Columbian and *buff* chicks in the ratio of 4: 3: 1; or approximately equal numbers of chicks with extended and restricted black pigment. When crossed with buff females the same F_1 males have produced chicks in approximately the ratio 2 black: 1 Columbian: 1 buff; again showing equality between the extended and restricted classes. The F_1 black females crossed with buff males have produced equal numbers of black and buff chicks, while the F_1 Birchen females have produced, when crossed with buff males, black, Columbian and buff chicks in a ratio approximating 2: 1: 1. The ratios as quoted above have all been obtained and will be reported in full when the adult classifications have been completed. All of these crosses represent matings of fowls heterozygous in extension (E^me^m) with fowls recessive in extension (e^me^m). The expectation is equal numbers of black (extended) and non-black (restricted) chicks. The experimental numbers at present are 99 black (E^m): 98 non-black (Columbian or buff e^m). A clear monohybrid segregation is evident between extension (E^m) and restriction (e^m) of black pigment.

The above results are all explained on the following hypotheses:

1. The black fowls have the dominant allelomorph of an autosomal gene (E^m) which determines the extension of black pigment to all parts of the plumage. The recessive allelomorph (e^m) of this gene is present in the Columbian and buff fowls.

2. The black fowls contain the recessive allelomorph of the dominant sex-linked gene S (silver). This dominant gene inhibits the production of buff ground color and causes the production of silver or white ground color;[1,2] it is known to be present in Columbian fowls, while the recessive allelomorph characterizes the buffs. As regards these two genes the blacks used in these experiments have proved to be E^mE^mss in composition, the Columbians e^me^mSS (male) or e^me^mS-(female), and the

[1] Sturtevant, A. H., 1912, *Jour. Exp. Zool.*, 12: 499–518.

[2] Dunn, L. C., 1922, AMER. NAT., 56: 242–255.

buffs $e^m e^m ss$. Blacks are therefore genetically buffs with an epistatic gene for the complete extension of black pigment.

3. Extension of black is incompletely epistatic over silver so that in fowls of the genotype $E^m e^m Ss$ (male) or $E^m e^m S$-(female) silver appears in certain parts of the plumage, producing a pattern like that of the Dark Brahma.

Collateral evidence indicates that the gene for extension of black pigment (or one with similar effects) is present in Barred Plymouth Rocks and White Plymouth Rocks (as a cryptomere); and that it is absent in Columbian and buff varieties and in Rhode Island Reds.

Hurst[3] was probably dealing with the same gene in his crosses between Black Hamburgs and Buff Cochins, since F_1 from this cross consisted of all black chicks, while in F_2 black and buff chicks occurred in the proportions of 88 black: 31 buff. In interpreting the results of this cross Morgan[4] states that either one or two pairs of factors may be involved; which is right "could only be determined by an F_2 ratio." Yet the F_2 ratio is given by Hurst (p. 138) and is surely a sufficiently close approach to a monohybrid ratio. The ratios obtained in our experiments agree throughout with a mono-factorial interpretation.

It is believed that this gene will be found to characterize many color varieties of fowls in which black, either as a self color or as a component of a pattern extends to all or nearly all of the plumage. Concerning its origin no direct evidence can be offered at present. Its occurrence as a discrete unit indicates, however, that its origin was discontinuous and that black varieties probably had their genesis in mutation rather than in selection of particolored types toward black.

L. C. DUNN

THE EFFECTS OF SO-CALLED CONJUGATION IN SHELLED RHIZOPODS[1]

THE phenomenon of conjugation in the Protozoa is regarded as the forerunner of sexual reproduction in the higher animals,

[3] Hurst, C. C., 1905, Reports to the Evo. Comm., II, pp. 138–39.

[4] Morgan, T. H., 1919, Publ. Carnegie Inst. No. 285, p. 24.

[1] The experimental work on this problem was carried on in the Zoological Laboratory of the Johns Hopkins University. I wish to thank Dr. H. S. Jennings, of that institution, for suggesting the problem and for his aid in pursuing the investigations.

in which there is a union of a sperm and an egg. This latter process is fundamental in the life of at least all the higher Metazoa. By this union the race is perpetuated and hereditary characters are intermingled. It would seem that studies of a similar process in the unicellular forms might throw light upon the basic relations of the sexual process to the life of protoplasm.

Various investigators have shown that conjugation is a relatively common occurrence among the more complex Protozoa, such as the Paramecium, and that hereditary characters are intermingled in this way. During conjugation two Paramecia fuse by their oral surfaces and there is an interchange of nuclear material between the individuals. The latter then separate and reproduction by division occurs. The races resulting from such divisions show the effects of modifications due to hereditary characters coming from both the conjugating individuals.

In the case of the more primitive Protozoa, the Amœba and other Rhizopods, not a great deal is known. It has been observed that sometimes two individuals unite, but it is uncertain whether or not true conjugation occurs with interchange of nuclear material and subsequent modification of the offspring. If such proves to be the case, we shall have shown that the phenomena of sex are found in the very lowest animals, and are of general fundamental importance in the life process.

The present investigation was undertaken in the effort to throw some light on this question. An attempt was made to test the matter by inducing conjugation between various individual Rhizopods and noting if any inherited differences arose from these unions. (A cytological study of the behavior of the nuclei of such individuals must be made before the evidence can be fully weighed.) A shelled Rhizopod, *Difflugia corona*, was used, since the shell exhibits marked characteristics which vary among the different races of the species. The ordinary shellless Amœba presents so few characters of a permanent nature that it is unsuited for a study such as this. The Difflugia is an Amœba which builds from microscopic sand grains a shell shaped much like an old-fashioned soap kettle with the legs represented by spines projecting from the rounded aboral surface. The animal lives inside this shell and thrusts its pseudopodia from the oral opening. It reproduces by division, as other Protozoa, half the body being extruded from the oral

opening of the shell and a new shell being formed over the exposed part of the body, the two shells, old and new, lying mouth to mouth. The body then divides and the new individual moves away to lead a separate existence.

At times two Difflugias have been observed attached mouth to mouth and have been thought to be conjugating. When dividing the new shell is much lighter in color than the old and in the cases supposed to be conjugating both shells were of the same shade, that is both dark, so that the phenomenon did not seem to be that of division. It was this attachment or so-called conjugation which I endeavored to investigate.

It was found by Dr. Jennings (not published as far as I am aware) that two Difflugias could sometimes be made to attach themselves together by keeping them in a drop of distilled water for a few hours. At his suggestion, I used this method, endeavoring to hasten the process somewhat by pushing the individuals together by means of a fine glass rod. In several cases, the members of the pair became quite firmly united and remained so for some time. These pairs I then put into a drop of culture medium in the concavity of a hollow ground slide. In some cases such individuals never separated but died. In several instances they did separate and lived and began to reproduce by division.

Briefly, my procedure then was this. The number of spines varies in different races of Difflugia, sometimes averaging 2 or 3, sometimes as high as 5 or 6. The size of the shell also varies. I used the diameter across the widest part of the shell. I selected, for example, a small individual with a few spines and kept it under observation on a hollow ground slide in culture medium until it had produced an offspring by division. (The culture medium consisted of tap water containing the microscopic débris washed from the leaves of Elodea.) I then isolated the offspring and allowed it to produce a race, keeping each individual separate on a slide, and measuring and counting the number of spines of each. The original Difflugia I tried to mate, so to speak, with a large Difflugia with many spines, which had previously produced an offspring which I had isolated and allowed to found a race. If the large and small Difflugias became attached, supposedly conjugating, and were successfully separated, I then isolated each one on a slide and allowed each to start a line of progeny. I then had 4 lines going, one from each Difflugia before conjugation and one from

each after conjugation with the other. The following diagrams may serve to make my meaning clear.

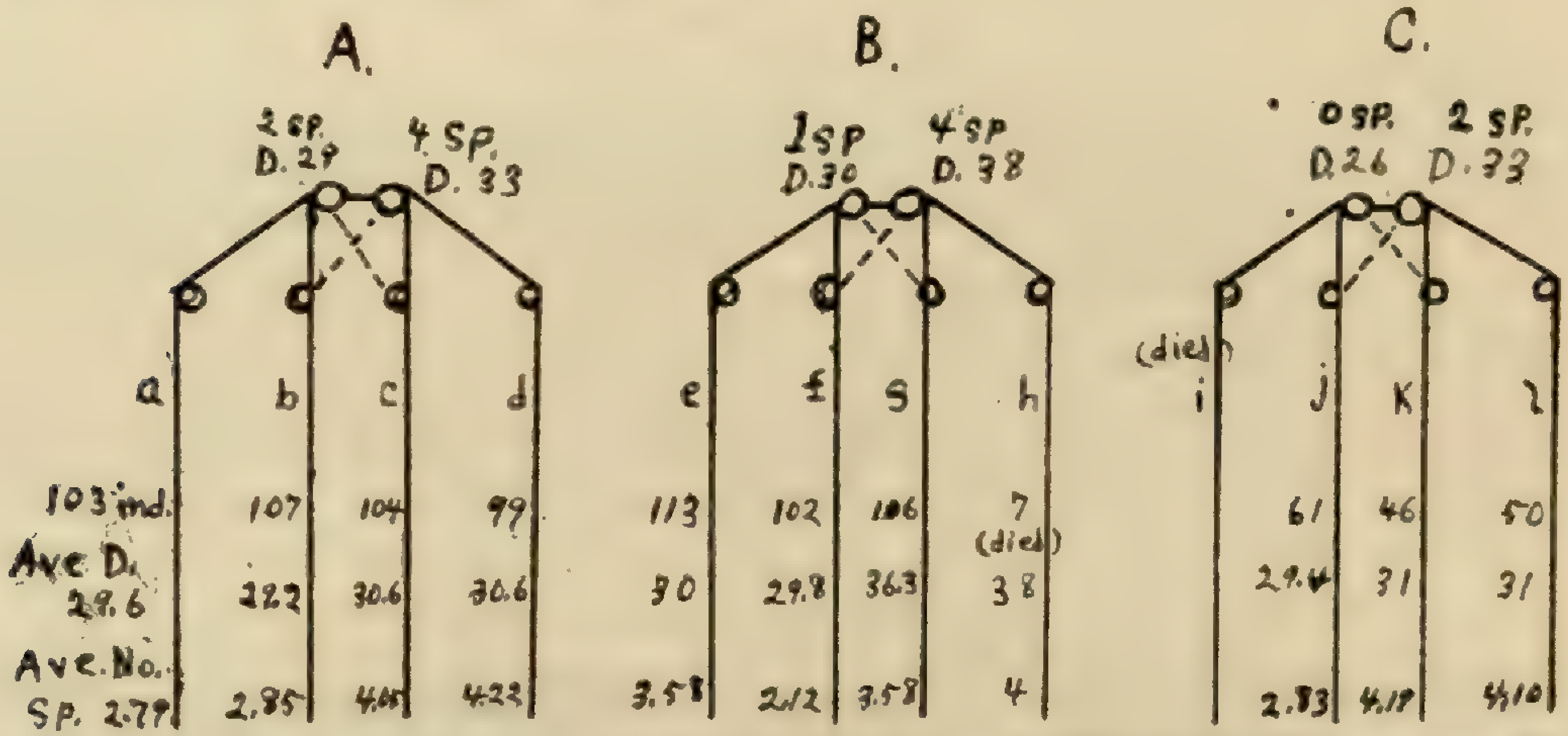

FIG. 1. Diagrams illustrating the manipulation of Difflugia in the experiments with so-called conjugation and the results attained in three cases. The two circles at the top of each diagram represent the individual Difflugias which became attached to each other. D., diameter. sp., spines. ind., individuals. Ave., average.

In each figure the circles at the top represent the Difflugia which were paired. For example, in *A* an individual with a diameter of 29 units founded a line *a* and then was paired with a Difflugia with 4 spines with a diameter of 33. This had previously started line *d*. After the separation of the Difflugias, they founded lines *b* and *c* respectively. The dotted cross lines represent the influence which might be expected to come from the other member of the pair if there were actual conjugation. Line *a* was carried on until 103 individuals were produced, the average number of spines being 2.79 and the diameter 29.6 units. Line *b*, started after the attachment of the progenitor to the other Difflugia, was carried for 107 individuals, with an average of 2.85 spines and a diameter of 29.2. The diagrams show the results gained from carrying out the lines in the other experiments. Unfortunately line *i* died before producing any individuals and line *h* produced only 7 before dying out.

By comparing the four lines in each experiment with each other, evidence may be gained relative to whether or not, during the supposed conjugation, the Difflugias exerted an effect upon each other sufficient to cause modification of the diameter and number of spines of the offspring in the direction of the line started by each before the attachment. That is, for example, line *b* should be more like line *d* than is line *a*, and line *c* should be more like *a* than is line *d*, and so on throughout the experiments.

A study of the data set forth in the diagrams will show that there is practically no modification of any line springing from a Difflugia, after the supposed conjugation, in the direction of the characters of the line founded by the other member of the pair before the union. Any slight changes seeming to show such modification are offset by just as marked variations away from that line. Comparing line *g* with *h*, it would seem that there is a marked modification of *g* in the direction of line *e*. It must be noted, however, that line *h* consists of only 7 individuals, so that the average is untrustworthy. In no other case is there any significant leaning toward the other line, although there are very slight tendencies in that way in the spine numbers in experiment A. Line *a* has 2.79 spines, *b* 2.85, *c* 4.05 and *d* 4.22. On the other hand, in experiment B the spine number in line *e* is 3.58 and in *f* only 2.12, to be compared with 4 in line *h*. It would be expected that line *f* would show a greater number of spines than line *e*. In experiment C line *k* shows a slight increase in spine number instead of the expected decrease. In general there are no apparent modifications of the offspring as the result of the pairing.

The experiments are open, of course, to the criticism that the attachment between the Difflugias was brought about by keeping the individuals in distilled water to bring them to the state of partial starvation which seems usually to be the forerunner of conjugation in the Protozoa. It is possible that under these somewhat unnatural conditions, the preliminary steps incident to conjugation would be inaugurated but that the process would stop before completion. In answer to this, I can only state that the Difflugias remained attached from 12 to 24 hours, thus allowing sufficient time for nuclear changes to have occurred. They became separated only when placed in cultures containing food.

To summarize then, as far as it is safe to base conclusions on the results of this limited number of experiments, it seems that the offspring of the Difflugias were not influenced by the attachment or so-called conjugation of the parents. From this fact it appears probable that this phenomenon of attachment sometimes observed in the shelled Rhizopods is not a true conjugation and that there is no interchange of nuclear material between the individuals taking part in it.

E. P. CHURCHILL, JR.

UNIVERSITY OF SOUTH DAKOTA

ON REARING SEXUALLY MATURE PLATYNEREIS MEGALOPS FROM EGGS

I

The literature affords so few cases of marine animals reared under laboratory conditions that the writer ventures to communicate his successful attempts to carry through to sexual maturity the nereid, *Platynereis megalops,* from eggs laid in the laboratory. This work had its origin in a suggestion made by Dr. F. R. Lillie in 1911 that the capacity for cross fertilization between *Nereis limbata* and *Platynereis megalops* be tested. At that time, however, since we knew so little of the life history of these forms, we felt that it was necessary to get all data possible on each life history in order to have a standard of comparison for the life history of the hybrids. So far all efforts to cross these nereids have failed. The difference in the breeding habits of *Nereis* and *Platynereis* is so striking that this alone might account for the failure of cross fertilization. *Nereis* sheds eggs into the sea-water where fertilization takes place; *Platynereis* lays inseminated eggs soon after copulation. However, this very difference is calculated to enhance the interest attaching to the cross fertilization. It might be possible to study the inheritance of the egg-laying reactions. In addition, early observations revealed that the young *Platynereis megalops* closely resemble *Nereis dumerilii.* Since, as is well known, *Nereis dumerilii* has a complex life history, we felt that the life history of *Platynereis* might well repay study for its own sake.

II

Platynereis megalops Reared under Laboratory Conditions to Sexual Maturity

The writer has found that it is possible to rear *Platynereis megalops* to sexual maturity under laboratory conditions. This was first accomplished in 1913–1914, repeated in 1920–1921, and again in 1921–1922. The results may be briefly recounted.

Methods

Males and females caught with a hand net in the evenings of the July and the August full moon are kept in separate dishes. In the laboratory as shortly after capture as possible a male and

a female are placed in a finger bowl of clean sea-water. After copulation and egg-laying the animals are removed from the finger bowl. After the jelly has been extruded by the eggs, the supernatant sea-water is poured off, leaving the eggs in the mat of jelly stuck to bottom of the bowl. At first cleavage, which is invariably one hundred per cent., the jelly-mass is gently broken up and the eggs equally distributed among seven to ten finger bowls of clean sea-water. Early the next morning the sea-water is changed. At this time all eggs that possess fewer or more than four oil drops, one in each macromere, are discarded. Only those larvæ that possess four oil drops evenly distributed among the four macromeres give rise to normal swimming larvæ. As the trochophores rise to the surface in each dish they are pipetted off. Trochophores that fail to swim at the surface in twenty-four hours lack the viability of those that rise earlier.

The young larvæ are kept in subdued light a few in each dish because they tend to aggregate in such dense masses that many die off. This tendency to collect in one spot makes it easy to change the water and thus avoid too great rise in temperature, which is fatal to the animals. The larvæ will reach the stage of three swimming segments without the addition of food.

When the segments appear, the larvæ must now be watched very carefully in order that food may be given at the proper time. *The criterion for the initial feeding is the complete disappearance of the oil drops from the entoderm cells.*

In the eggs of both *Nereis* and *Platynereis* there is at the time of fertilization a girdle of some eighteen to twenty-two oil drops in the equatorial zone. These oil drops in the maturation stages following insemination move to the vegetative pole. During cleavage the number of oil drops is reduced to four large globules which normally are distributed to the cells of the gut. Beginning with the third or fourth day after laying, the oil in the gut cells of the larvæ begins to form an emulsion of smaller and smaller drops. It is thus possible to follow the history of the oil drops very fully in these creatures that make veritable living test tubes in a fat-digestion experiment. If food is given the worms before the oil has been completely used, they are killed in large numbers. On the other hand, food must not be withheld too long after the disappearance of the oil. The first feeding consists of ten c.c. of a diatom culture known by previous examination under the microscope to be free of metazoa or larvæ,

strained through three thicknesses of bolting silk of very fine mesh. As the larvæ add segments more food is given. When the larvæ build their tubes both food and mud are added until the bottom of the dish is well covered. The method of preparing the diatom culture may now be considered.

In 1911 I procured food according to the method described by Hempelmann in his study of the life history of *Nereis dumerilii* at Naples. This method consists principally in scraping the growths from the live tables. The bottom and sides of aquaria under running sea-water frequently show a felt-like growth of diatoms and protozoa. Scrapings from such aquaria suspended in sea-water will give food sufficient to keep a few young worms of both *Nereis limbata* and *Platynereis*. The method does not allow the rearing of any large number of worms. Similarly, attempts at a pure culture of diatoms gave poor results.

In 1913 through the kindness of Dr. Caswell Grave I procured a remarkably fine culture of diatoms from Beaufort, N. C. With this, the first sexually mature worms were obtained. But obviously, *Platynereis* at Woods Hole must live on food got in Woods Hole waters. I, therefore, made various attempts to get an adequate diatom culture from the immediate vicinity. The successful method follows.

At the beginning of the season mud is taken from Eel Pond, near-by flats, or scraped from eel grass, together with animal and plant life. This is placed in jars with the addition of an equal volume of sea-water. The jars are then covered with glass plates and set aside in subdued light. In a day or so all metazoa—worms, crustacea, ascidians, etc.—are dead. After a period of putrefaction, the culture purifies itself and a rich growth of diatoms begins.

For young worms a suspension of diatoms strained through several thicknesses of bolting silk is used. The diatoms for this purpose are previously examined under the microscope, one c.c. at a time; usually no metazoa are found. The suspension is then made up in filtered sea-water. As the larvæ increase in size and vigor food is added in greater quantities.

A brief summary of the three cultures of *Platynereis megalops* reared to sexual maturity may now be given.

The Larval Cultures

The 1911 and 1912 larvæ were not kept after September first.

The 1913 Culture.—During August, 1913, from eggs laid in

the laboratory by twenty females over 100,000 larvæ were reared. On the eve of my departure from Woods Hole the larvæ were all carefully removed from their tubes and placed in half-gallon Mason jars. Each jar contained 500 c.c. of the rich Beaufort diatom culture and sea-water to within ten centimeters of the top. The jars were then tightly covered and set aside. After the worms had had time to build new tubes, the jars were shipped to Washington, D. C. The worms at this time averaged about 10 mm. in length. Early in June, 1914, these worms were shipped from Washington to Woods Hole. Relatively few survived the journey. The largest worms (females), brought carefully in a hand bag, died before the journey was half made. Since during the winter hundreds of these worms had been killed periodically for future study, the number left from the 1913 culture had been greatly reduced. Some animals of this culture were carried through the summer of 1914. They never reached sexual maturity. They were taken back to Washington at the end of 1914. In 1915 they were brought back to Woods Hole and returned to Washington that fall. During this period they still showed no change.

In Washington the animals were kept in the clamped jars without any change of water or additions of distilled water. One culture was kept in a battery jar covered with a glass plate. Nor was any addition ever made to this culture. The jars were kept at room temperature in subdued light. To avoid contamination worms removed for study were from the culture in the battery jar only. After observing the worm I never replaced it, but killed it for *in toto* mount or sectioning.

In 1917 several very fine cultures of worms were started, but they died in transit to Washington. In 1918 and 1919 no worms were reared.

The 1920 Culture.—In August, 1920, very beautiful cultures of about 50,000 larvæ were started: unfortunately, the majority of these died very suddenly late in August. About a thousand worms survived. These were distributed among twelve dishes with food and left over winter in the heated laboratory at Woods Hole. The dishes were left covered with glass plates exposed to north light. No change was made in the water or any additional food given during the period September 1, 1920, to June 1, 1921. On May 17, 1921, about 200 worms of different sizes were found in the dishes. Of these some were preserved from time to time.

The history of the others shows that the first female with ripe eggs appeared June 5. She was discovered slowly crawling around the dish near the surface of the water. In color and in form this animal resembled the females collected during the breeding season. In size she was rather below the average and somewhat more sluggish. The eggs in size, color and form were identical with the eggs got from animals captured during the breeding season. Subsequently, mature females were found at intervals through the summer. No males appeared until late in June. Eventually, thirty-two females and twelve males fully mature were got from this 1920 culture.

Males got from the sea copulate with females reared in the laboratory; such females lay normal eggs that give rise to larvæ of a high degree of viability. Males reared in the laboratory copulate with females taken from the sea. The eggs are perfectly normal. On only one occasion did I find a male and female from this 1920 culture sexually mature at the same time. They copulated in normal fashion. The eggs laid were normal in every respect and gave rise to larvæ that I kept for two weeks before discarding. These larvæ could not be distinguished from larvæ resulting from eggs laid by animals taken from the sea.

The 1921 Culture.—At this writing only two mature animals (females) have appeared—one May 1 and one May 6, 1922.[1]

The Rate of Growth

Some idea of the rate of growth in these worms may be obtained from data collected from the 1913 culture. This was the only culture on which I had the opportunity to make continuous observations.

Life History

Observations so far made on cultures of *Platynereis megalops* reared in the laboratory from eggs laid by animals taken from the sea do not reveal any indication of a sexually mature intermediate form. So far, all eggs obtained appear to be identical with those got from animals in nature. This would seem to suggest that the life history of *Platynereis* is simple—without the complexity of form and sexual condition found in *Nereis dumerilii*, which *Platynereis* so closely resembles. It must be clearly

[1] Since the above was written, 23 animals have reached maturity—17 females and 5 males. One reason for this sex ratio is that the males have difficulty in getting out of their tubes; their mortality is therefore high.

stated, however, that on this point the observations so far made are not conclusive. In order to determine fully that the eggs laid by worms in the cultures in the laboratory are from the same worms started in the culture and not from an intermediate form and are the only eggs laid, it would be necessary to make continuous observations on isolated worms. So far it has not been feasible to do this, since it would mean practically continuous residence at Woods Hole through the winter.

TABLE I

RATE OF GROWTH OF PLATYNEREIS MEGALOPS FROM EGGS LAID ON THE EVENING OF JULY 21, 1913

Date		No. of Segments with Parapodia	Length in Mm.
July	28, 1913	3	
July	29	4 to 5	
July	30	5	
July	31	6	
August	2	10	
August	6	16	2
August	8	22	4
August	12	26	5
August	26		7
Sept.	15		14
Oct.	1		18
Nov.	1		20
Dec.	1		25
Jan.	10, 1914		30
Feb.	2		33
March	1		40–45
April	3		40–50
May	6		50–60
May	28		40–50

On the other hand, it is just barely possible that in a state of nature the life history is more complex than in the laboratory cultures. Under operation of changes in such factors as density of the sea-water, food, and temperature, the life history of the worms may be modified. That this possibility deserves some consideration we may conclude from the sex ratio, if such meagre data will allow. In the laboratory cultures females appeared first in all three years and they outnumber the males. In nature just the reverse is true.

Whatever our conclusions as to the interpretation of these observations, it seems to the writer that the life history of this interesting nereid is worthy of further study.

A Comparison with Other Forms

The method used for rearing sexually mature *Platynereis* from the fertilized egg has been used to rear other worms through to the adult stage: namely, *Pectinaria gouldii, Diopatra, Nereis limbata,* and *Chætopterus.* In all cases the worms were reared from eggs cut out of the females and inseminated in sea-water. In no case were the worms kept beyond September 15 (from one to three months). Though it is usually stated that artificial insemination of *Diopatra* eggs is not possible, every attempt made by the writer in 1911, 1912, 1913, 1914 and 1915 was successful. There is one danger to avoid with these eggs—initiation of development by mechanical shock. The worms reared from *Diopatra* eggs are if anything more hardy than those of *Platynereis.* In 1913 I reared *Diopatra* in a watch glass to a length of four centimeters.

Pectinaria gouldii are likewise readily reared from eggs inseminated in the laboratory. These eggs are extremely beautiful, small, and almost wholly transparent. They are easy to handle. I have found them the best eggs in my experience for study under high power (oil immersion lenses).

The specimens used were from the Eel Pond and are normally smaller than *Pectinaria* found outside of Eel Pond. They are infested with a distome and an interesting ciliate; the latter I did not find in the larger specimens (1911). This, if it be generally true, together with the size of the Eel Pond specimens makes an interesting case from the point of view of ecology.

Among the shed spermatozoa of *Pectinaria* are many in bundles that break up after a short time in the sea-water. In addition to these one can always get bundles of spermatocytes, immature sperm, etc., by puncturing the body wall. It is a very excellent form to use for the study of cytoplasmic inclusions: it is possible to get the whole history of the sperm on one slide.

My object in studying these ova was to try to learn if size, opacity, and yolk influence the ease with which the animals can be reared under laboratory conditions. I found no correlation. Thus, the egg of *Platynereis* is almost transparent; it measures 180–200 μ. *Nereis* egg has more color and measures about 100 μ. The *Nereis* egg is the hardest of all to carry through. The egg of *Pectinaria* is small and almost wholly transparent. It is readily reared. The *Chætopterus* egg has more color than that of *Nereis* and is smaller. It is easier to rear than the egg of *Pectinaria.* The *Diopatra* egg is wholly opaque; it is the largest

of the five eggs and perhaps the easiest to rear. The eggs may be arranged according to size, depth of color and ease with which they may be reared as follows:

Size	Depth of Color	Ease of Rearing
Diopatra	*Diopatra*	*Diopatra*
P'atynereis	*Chætopterus*	*P atynereis*
Nereis	*Nereis*	*Chætopterus*
Chætopterus	*P atynereis*	*Pectinaria*
Pectinaria	*Pectinaria*	*Nereis*

As in the case of *Platynereis* the essential point in rearing these annelids is to give them food at just the right time in the larval stage. This time varies somewhat with each form. Briefly, food must not be given before the yolk and oil are wholly used up. One needs but to watch the larvæ, note the disappearance of the oil from the gut, and then add diatoms.

E. E. JUST

HOWARD UNIVERSITY

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AN OBSERVATION ON THE "CLUSTER-FORMATION" OF THE SPERMS OF CHITON[1]

WHILE engaged with an inquiry into the natural history of the chitons, in 1918,[2] I several times made an observation which may have a bearing on the significance of sperm-clusters, and on the mechanism of their formation. The matter could not at the time be adequately investigated, but since I shall not soon be in a position to examine it further my observations are here related for what they may be worth. The species concerned is *Chiton tuberculatus* Linn., an intertidal form quite abundant at Bermuda. It is necessary to note, first, certain features of the breeding process, which seems to me to have heretofore been

[1] Contributions from the Bermuda Biological Station for Research. No. 119.

[2] The corrected proof and manuscript of this article were returned to the publisher Aug. 18, 1920; but the corrected article was accidentally taken out of type in the office of the printers. The author has now re-written the paper. E. L. M.

somewhat misunderstood. In another connection I shall describe several aspects of the reproductive activities of these animals, the present remarks having to do merely with the act of fecundation.

Although it has commonly been held that the liberation of eggs by a female chiton is due to the reception of spermatic fluid diffusing into her respiratory water-currents from a near-by male, the process of fertilization would appear in fact to be initiated in a quite different manner. Stated briefly, the presence of one or more neighboring females serves in some way to activate the discharge of sperm by the males, the spermatic substances secondarily inducing the liberation of eggs. Normally this occurs only at those periods when the flow of the tide begins just before sunrise, the shedding of the genital products commencing as the chitons become covered by the sea. The discharge of sperm can, however, be induced artificially at certain times, in the laboratory, even a month or more before the eggs are matured.[3] A method which several times yielded this result consisted in keeping some male chitons in a damp, darkened vessel for about 14 hours, then covering them with sea water and admitting light. It should be noted here that *C. tuberculatus* is an animal nicely fitted for observations of this kind, because the differential pinkish tint of the soft tissues of the females permits the quick and accurate identification of sex.[4]

In May, a month before ripe eggs are seen, it was noticed that when sperm diffusing from a male, in a glass dish, was taken up between the ctenidia of a female, it issued from the posterior ends of the ctenidial channels in an altered state, for the sperm-stream was then seen to contain numerous agglutinated masses of active sperms, which persisted in sea water for at least half an hour.

During natural fecundation, however, no sperm-balls are formed. The thick glutinous stream of spermatozoa passes under the girdle of a female, is somewhat diluted with sea water

[3] That the discharge of sperm is under nervous control is indicated by the behavior of male *Chætopleura* following strychninization (cf. Crozier, 1920, *Jour. Gen. Physiol.*, Vol. 2, pp. 627–634).

[4] See Crozier, W. J., 1920, "Sex-correlated Coloration in Chiton," AMER. NAT., Vol. 54, pp. 84–88. Tidal, or rather lunar, periodicity in the liberation of gametes has been observed also in *Chætopleura;* I was able to note a probable lunar periodicity in this genus, in 1919, at Woods Hole, and the point is dealt with at length in a recent paper by Grave, B. H., 1922. *Biol. Bull.*, Vol. 42, pp. 234–256.

by the tractive current, and emerges posteriorly in company with numerous large greenish eggs, about which, under the microscope, it can be seen that many sperms are gathered. But no real "cluster-formation" takes place.

The body juices of the ripe female, whether or not diluted with sea water, do not cause agglutination of sperm suspensions. But ovarian extracts from (*mature*) eggs in sea water do induce decided and apparently typical agglutination. So far as I know, sperm-agglutination by ovarian extracts has not previously been seen in molluscs.[5] Sea water into which ripe eggs have been shaken from an ovary and the whole allowed to stand for half an hour has a similar agglutinative effect.

Concerning the significance of the cluster formation, then, these two points seem significant: (1) the absence of such a process in normal fecundation, and (2) its conspicuous occurrence when sperm, before the real onset of the breeding season, has passed through the ctenidial channels of males or immature females. It could not be discovered whether or not the *mature* female in a non-spawning interval would cause this cluster production, because at such times the consistent response of a female to an impinging current of sperms was to depress the girdle to the substratum, thus cutting off the water current carrying sperms, and, by reducing the volume of the ctenidial channel, violently to expel from below the sperms already admitted.

These observations do not, of course, permit analysis of the rôle of egg-substances in fertilization of chiton, but do serve to point the contention that mere evidence of sperm agglutination (cluster formation) may well have no bearing on such analysis. It is possible that the sperms set free at a period before the natural ripening of eggs are in some degree immature, their surface perhaps more sticky, or liable to be made so by slight external changes experienced in passing between the gill filaments of another individual.

W. J. CROZIER

ZOOLOGICAL LABORATORY,
RUTGERS COLLEGE

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THE PROGRESSION OF LIFE IN THE SEA[1]

DR. E. J. ALLEN, F.R.S.

THE method we usually follow in the ordinary course of zoological work is to make first, with the unaided eye, a general examination of the animal that interests us, and then study in detail its separate parts with a simple lens, with a low power of the microscope, with gradually increasing powers, until, finally, minute portions are examined with the highest oil-immersion lens. The successful research worker is generally one who, whilst carrying to the utmost limit he can achieve his search into detail, maintains as by instinct a true sense of proportion and holds firmly to the idea of the organism as a whole.

In discussing the living organisms of the sea I shall try to follow a similar plan, thinking of the life of the sea as a whole, built up of individual plants and animals, each in intimate relation with its surroundings, and all interdependent among themselves. But even this is not enough, for we must take still a wider view and keep in mind not only the life of the waters, but that also of the land and of the air, for both, as we shall see, have a bearing on our theme. Deep oceans, coastal waters, shallow seas, rivers and lakes, continents and islands, all play their part in one scheme of organic life—life which had, it seems, one origin, and, notwithstanding migrations and transmigrations from water to land, from land to air, and from land and air back again to the water, remains one closely interrelated whole.

[1] Address of the President of the Section of Zoology of the British Association for the Advancement of Science, Hull, September, 1922.

Both Brandt[2] and Gran[3] have recently emphasized the fact that it is in the coastal waters and shallow seas, which receive much drainage from the land, that plant and animal life are most abundant, the more open oceans far from land being relatively barren; as Schütt puts it, the pure blue of the oceans is the desert color of the seas. This increased production in the coastal waters is due principally to the presence of nitrogen compounds and compounds of phosphorus derived from terrestrial life. From forest, moor and fen, wherever water trickles, the life of the land sends its infinitesimal quota of these essential foodstuffs to fertilize the sea.

When, however, we go back to the beginning of things, we shall probably be right if we say that any influence of terrestrial life upon life in the sea must be left out of account. Different views are still held as to where life in the world had its origin, but no one questions that it began in close connection with water. That it began in the sea, where the necessary elements were present in appropriate concentrations and in an ionized state, is an idea which appeals to many with increasing force the more closely it is examined. This view has been developed recently by Church[4] in his memoir on "The Building of an Autotrophic Flagellate," in which he boldly attempts to trace the progression from the inorganic elements present in sea-water to the unicellular flagellate in the plankton phase, floating freely in the water. The autotrophic flagellate, manufacturing its own food, he regards as the starting-point from which all other organisms, both plants and animals, have sprung. To understand the first step in this progression, the passage from the dead inorganic to the living organic remains, as it has always been, one of the great goals of science, not of biological science alone, but of all science. Recent research has, I think, thrown much light on the fundamental problems involved. In a paper published last year, Baly,

[2] *Wissensch. Meeresunters.* Kiel, 18, 1916–20, p. 187.

[3] *Bull. Planktonique.* Cons. Internat., 1912 (1915).

[4] *Biological Memoirs I.* Oxford, 1919.

Heilbron, and Barker,[5] extending and correcting previous work by Benjamin Moore and Webster,[6] have shown that light of very short wave-length ($\lambda = 200\,\mu\mu$), obtained from a mercury-vapor lamp, acting upon water and carbon dioxide alone, is capable of producing formaldehyde, with liberation of free oxygen. Light of a somewhat longer wave-length ($\lambda = 290\,\mu\mu$) causes the molecules of formaldehyde to unite or polymerize to form simple sugars, six molecules of formaldehyde, for example, uniting to form hexose. The arresting fact brought out in these researches is that the reactions take place, under the influence of light of appropriate wave-lengths, without the help of any catalyst, either organic or inorganic. Where a source of light is used which furnishes rays of many wave-lengths, the simple reaction of the formation of formaldehyde is masked by the immediate condensation of the formaldehyde to sugar, but this formation of sugar can be prevented by adding to the solution a substance which absorbs the longer wave-lengths, so that only the short ones which produce formaldehyde are able to act.

When the formation of sugars is postulated, the introduction of nitrogen into the organic molecule offers little theoretical difficulty; for not only has Moore[7] shown that nitrates are converted into the more chemically active nitrites under the influence of light of short wave-length, but he maintains that marine algæ, as well as other green plants, can under the same influence assimilate free nitrogen from the air. Baly[8] also has succeeded in bringing about the union of nitrites with active formaldehyde in ordinary test-tubes by subjecting the mixture to the light of a quartz-mercury lamp.

It will be admitted that these three reactions: (1) the

[5] *Journ. Chem. Soc.*, London, Vols. 119 and 120, 1921, p. 1025. *Nature*, Vol. 109, 1922, p. 344.

[6] *Proc. Roy. Soc.* B., Vol. 87, p. 163 (1913), p. 556 (1914); Vol. 90, p. 168 (1918).

[7] *Proc. Roy. Soc.* B., Vol. 90, p. 158 (1918); Vol. 92, p. 51 (1921).

[8] Baly, Heilbron and Hudson, *Journ. Chem. Soc.*, London, Vols. 121 and 122, 1922, p. 1078.

formation of formaldehyde, H.CO.H, from carbonic acid, OH.CO.OH, with liberation of free oxygen, or, to put it more simply, the direct union of the carbon atom of CO_2 with a hydrogen atom of H_2O; (2) the formation of sugars from formaldehyde, and (3) the formation from nitrites and formaldehyde of nitrogenous organic substances, are the most fundamental and characteristic reactions of organic life. It is true that light of such short wave-lengths ($\lambda = 200\ \mu\mu$) as were required in Baly's experiments to synthesize formaldehyde does not occur in sunlight as it reaches the earth to-day; but, as we shall see later, the same author has shown that, in the presence of certain substances known as photocatalysts, the reaction can be brought about by ordinary visible light; and from Moore and Webster's work it appears that colloidal hydroxides of uranium and of iron are suitable photocatalysts for the purpose.

If these results of the pure chemist are justified, they go far towards bridging the gap which has separated the inorganic from the organic, and make it not too presumptuous to hazard the old guess that even to-day it is possible that organic matter may be produced in the sea and other natural waters without the intervention of living organisms. We may note here, too, that if we take account of only the most accurate and adequately careful work, the actual experimental evidence at the present time requires the presence of a certain amount of organic matter in the culture medium or environment before the healthy growth of even the simplest vegetable organism can take place. This was illustrated in some experiments made by myself some years ago when attempting to grow a marine diatom, *Thalassiosira gravida,* in artificial sea-water made up from the purest chemicals obtainable dissolved in twice-distilled water. Even after nutritive salts, in the form of nitrates and phosphates, had been added, little or no growth of the diatom occurred. But if as little as 1 per cent. of natural sea-water were added, excellent cultures resulted, in which the growth was as healthy and vigorous as I was able to obtain when natural sea-water was

used entirely as the basis of the culture medium. There was clearly some substance essential to healthy growth contained in the 1 per cent. of natural sea-water, and from further experiments it became practically certain that it was an organic substance. When, for instance, the natural sea-water was evaporated to dryness, the residue slightly heated and redissolved in distilled water, 1 per cent. of this solution added to the artificial culture medium was as potent in producing growth of the diatom as the original natural sea-water had been. When, on the other hand, the residue after evaporation was well roasted at a dull red heat and redissolved in distilled water, the addition of this solution to the artificial culture medium produced no effect and growth did not take place. Growth could also be stimulated by boiling a small fragment of green seaweed (*Ulva*) in the artificial culture medium, the weed being removed before inoculation with the diatom. All this points to the necessity for the presence of some kind of organic matter in the solution before growth can take place. One must not dogmatize, however, for there are many pitfalls in the experimental work and the necessary degree of accuracy is difficult to attain. My own experience of these difficulties culminated when I discovered, covering the bottom of my stock bottle of distilled water—water which had been carefully redistilled from bichromate of potash and sulphuric acid in all-glass apparatus—a healthy growth of mold.

Let us then assume that we are allowed to postulate in primitive sea-water or other natural water organic compounds formed by the energy of light vibrations from ions present in the water, and see how we may proceed to picture the building up of elementary organisms. Without doubt the evolutionary step is a long and elaborate one, for even the simplest living organism is already highly complex both in structure and in function. As the molecules grew more complex by the progressive linkage of the carbon atoms of newly formed carbohydrate and nitrogenous groups, we must suppose that the organic substance, for purely physical reasons, assumed

the colloidal state, and at the same time its surface-tension became somewhat different from that of the surrounding water. With the assumption of the colloidal state, the electric charges on the colloidal particles would produce the effect of adsorption and fresh ions would be attracted from the surrounding medium, producing a kind of growth entirely physical in character. We thus arrive at the conception of a mass of colloidal plasma differing in surface-tension from the water and increasing in size by two processes, the one chemical, due to linkage of carbon atoms; the other physical, brought about by the adsorption of ions by the colloidal particles.

The difference of surface-tension would tend to make the surface a minimum and the shape of the mass spherical. On the other hand, maximum growth would demand maximum exchange with the surrounding medium, and hence maximum surface. From the antagonism of these two factors, surface-tension and growth, there would follow, firstly, the breaking up of the mass into minute particles upon the slightest agitation, and, secondly, changes of form wherever growth involved local alterations of surface-tension, which changes of form would represent the first indication of the property of contractility.

So far we have considered only the process of the building up of the elementary plasmic particles, the anabolic process. Church, whose memoir already referred to I am now closely following, points out that these anabolic operations must from the beginning have been subject to the alternations of day and night, for during the night the supply of external energy is removed. "If during the night," he asks, "the machine runs down, to what extent may it be possible so to delay the onset of molecular finality that some reaction may continue, at a lower rate, until the succeeding day?" And his answer is: "The successful solution of this problem is defined physiologically by the introduction of the conception '*katabolism*,' as implying that energy derived from the 'breaking down' of the plasma itself . . . may be regarded as a 'secondary engine,' functional in the absence

of light, and evolved as a last resort in failing plasma." Katabolism persists as the ultimate mechanism in the physiology of animal as contrasted with plant life, but if the suggestion just quoted is sound, it originated, as the first "adaptation" of the organism, to meet the factor of recurring night and day. That the problem was successfully solved we know, but as to the mechanism of its solution we have no key. It is at this point again, to use Church's words, that the "plasma, previously within the connotation of chemical proteid matter, becomes an autotrophic, increasingly self-regulated, and so far individualized entity, to which the term 'life' is applied."

The elementary plasma is thus now fairly launched as an individual living organism, and the great fundamental problems of biology—memory, heredity, variation, adaptation—face us at each step of our further progress. We see in broad outline the conditions the advancing organism had to meet, we see the means by which those conditions were in fact met, we know that only those individuals survived which were able to meet them. Further than this we, the biologists of to-day, have not advanced. The younger generation will pursue the quest, and, with patient effort, much that now lies hidden will grow clear.

The differentiation of the growing particles of plasma into definite layers, which followed, seems natural; first the external layer, in molecular contact with the surrounding water, from which it receives substances from outside in the form of ions, and to which it itself gives off ions; beneath this the autotrophic layer to which light penetrates, and in which, under the influence of the light, new organic substance is built up; in the center a layer to which light no longer penetrates. This central region, the nucleus, depends entirely on the peripheral layers for its own nutrition, and becomes itself concerned only with katabolic processes, those processes of the organism which depend upon the breaking down, and not the building up, of organic substance.

At an early stage in the development of the individual organism the spherical shape, which the organic plasma

was compelled to assume under the influence of surface-tension, underwent an important modification, the effect of which has impressed itself upon all later developments. A spherical organism floating in the water and growing under the direct influence of light would obviously grow more rapidly on the upper side, where the light first strikes it, than it would on the lower side away from the light. There followed, therefore, an elongation of the sphere in the vertical direction, and the definite establishment of an anterior end, the upper end which lay towards the light and at which the most vigorous growth took place. In this way there was established a definite polarity, which has persisted in all higher organisms, a distinction between an anterior and a posterior end. With the concentration of organic substance which took the form of nucleus and reserve food supply, the specific gravity of the plasma would become greater than that of the surrounding water and the organism would tend to sink. The necessity, therefore, arose for some means of keeping it near the surface, that it might continue to grow under the influence of light. The response to this need, however it was attained, came in the development of an anterior flagellum. This we may regard as an elongation in the direction of the light of a contractile portion of the external layer, moving rhythmically, which by its movement counteracted the action of gravity, and acting as a tractor drew the primitive flagellate upwards towards the surface layers, into a position where further growth was possible. That this speculation of Church's represents what was actually accomplished, even though it does not make clear the means by which it was brought about, is shown by the interesting researches of Wager[9] on the rise and fall of the more highly organized flagellate *Euglena*. *Euglena* is a somewhat pear-shaped flagellate, the tapering end being anterior and provided with a single flagellum, which acts as a tractor drawing the organism towards the light. The

[9] *Phil. Trans. Roy. Soc.*, Vol. 201, 1911; and *Science Progress*, Vol. vi, October, 1911, p. 298.

posterior end carries the nucleus and most of the chlorophyll and food reserves. The whole organism has a specific gravity of 1.016, being slightly heavier than the fresh water in which it lives. When dead, or when the flagellum is not moving, it takes up, under the action of gravity alone, a vertical position in the water, with the pointed anterior end uppermost, and the heavier, rounded, posterior end below, and sinks gradually to the bottom.

In a very crowded culture a curious phenomenon is seen, because the organisms tend to aggregate into clusters beneath the surface film, and when they are crowded together in these clusters the flagella cease to work. This makes the whole cluster sink to the bottom under the action of gravity. When the bottom is reached the individuals are spread out by the action of the downward current, and, when they are sufficiently widely apart, the flagella again begin to move, carrying the organisms in a more diffuse stream once more to the surface. The whole culture vessel becomes filled with a series of vertical lines of closely aggregated falling organisms, surrounded by a broad cylinder of disseminated swimming ones, rising to the surface by the action of their flagella. If the conditions are kept uniform, such a circulation of *Euglenas*, falling to the bottom by gravity when the flagella are stopped and returning to the surface under their own power, will continue for days.

The flagellum in this species, therefore, retains its most primitive function of drawing the organism to the light in the surface layer. With the establishment of the flagellum an organ is produced which shows remarkable persistence in both the animal and vegetable kingdoms, and from the existence of the flagellated spermatozoon in the higher vertebrates, in accordance with Haeckel's biogenetic law that the individual in its development repeats or recapitulates the history of the race, we conclude that they also in their earliest history passed through a plankton flagellate phase.

Exactly at what stage in the history of the autotrophic flagellate the first formation of chlorophyll and its allied

pigments took place we have no means of determining, but it may have been before even the flagellum itself had begun. This advance and the subsequent concentration of the pigments into definite chromatophores or chloroplasts doubtless immensely increased the efficiency of the organism in producing the food which was necessary to it. The recent work of Baly and his collaborators becomes here again of the first importance, and though the subject of the part played by chlorophyll in photosynthesis belongs rather to botany and chemistry than to zoology, I may perhaps for the sake of completeness be allowed to refer to it very briefly. I have already said that Baly brought about the synthesis of formaldehyde from CO_2 and H_2O under the influence of rays of very short wave-length ($\lambda = 200\,\mu\mu$) from a mercury-vapor lamp. He was also able to show that when certain colored substances were added to the solution of carbon dioxide in water the same reaction took place under the influence of ordinary visible light. His explanation of this process is that the colored substance known as the photocatalyst absorbs the light rays and then itself radiates, at a lower infra-red frequency corresponding to its own molecular frequency, the energy it has absorbed. At this lower frequency the energy thus radiated is able to activate the carbonic acid, so that the reaction leading to the formation of formaldehyde can and does take place. In the living plant this synthesized formaldehyde probably at once polymerizes to form sugars.

Malachite green and methyl orange, as well as other organic compounds, were found to act as photocatalysts capable of synthesizing formaldehyde, and Moore and Webster's work had previously shown that inorganic substances, such as colloidal uranium oxide and colloidal ferric oxide, can do the same. Chlorophyll in living plants may with some confidence be assumed to operate in a similar way, though no doubt the series of events is more complex, since the green pigment itself is not a single pigment, and others, such as carotin and xanthophyll, are also concerned.

We have tried to picture the gradual building up from elements occurring in sea-water of a chlorophyll-bearing flagellate, capable of manufacturing its own nourishment and able to multiply indefinitely by the simple process of dividing in two. If we assume only one division during each night as a result of the day's work in accumulating food material, such an organism would be able in a comparatively short space of time to occupy all the natural waters of the world. But here we are met by a difficulty which is not easily overcome. Chlorophyll, the photocatalyst, the most essential factor in the building up of the new organic matter, is itself a highly complex organic substance, and in any satisfactory theory its original formation and its constant increase in quantity must be accounted for. Lankester[10] has maintained that chlorophyll must have originated at a somewhat late stage in the development of organic life, and has suggested that earlier organisms may have nourished themselves like animals on organic matter already existing in a non-living state. An alternative hypothesis, which in view of the recent work seems more attractive, is to suppose that the earlier organisms were either activated by some simpler photocatalyst, or that they received the necessary energy at suitable frequency directly from some outside source.

It must not be forgotten, also, that at the time these developments were taking place the conditions of the environment would in many ways have been different from those now existing in the sea. One suggestion of special interest that has been made[11] is that the concentration of carbon dioxide in the atmosphere, and hence also in natural waters, was very much greater than it is to-day. Free oxygen, indeed, may have been entirely absent, and all the free oxygen now present in the air may owe its existence to the subsequent splitting up of carbon dioxide by the action of plant life. With such possibilities of differences in the conditions in this and in so many

10 "Treatise on Zoology," Part I, Introduction. London, 1909.

11 See Carl Snyder, "Life without Oxygen," *Science Progress*, Vol. vi, 1912, p. 107.

other directions, may we not be well satisfied if, for the time, we can say that the formation of carbohydrates and proteids has been brought within the category of ordinary chemical operations, which can occur without the previous existence of living substance?

To return once more, however, to the free-swimming, autotrophic flagellate. In the early stages of its history the loss caused by sinking, and so getting below the influence of light and the possibility of further growth, must have been enormous. We may conceive a constant rain of dead and dying organisms falling into the darker regions of the sea, and thus a new field would be offered for the development of any slight advantages which particular individuals might possess. Under such conditions we may suppose that the holozoic or animal mode of nutrition first began in the absorption of one individual by another one, with which it had chanced to come into contact. If the one individual were more vigorous and the other moribund, we should designate the process "feeding," and the additional energy obtained from the food might well cause the individual to survive. If the two individuals which coalesced were both sinking from loss of vigor, the combined energy of the two might make possible a return to the upper water layers, where under the influence of light growth and multiplication would proceed, and we should, I suppose, designate the coalescence "conjugation," or sexual fusion.

Other individuals, again, sinking in shallow water, would stick to solid objects on the sea-floor, whilst the flagellum continued to vibrate. The current produced by the flagellum under these conditions would draw towards the organism dead and disintegrating remains of its fellows, and again we should have ingestion and animal nutrition. At this stage we witness the definite passage from plant to animal life. A further stage is seen when a cup-like depression to receive the incoming particles of food is formed at the base of the flagellum, to be followed still later by a definite mouth.

Any roughening of the external surface of the swim-

ming flagellate, such as we so often find brought about by the deposition of calcareous plates or silicious spicules or the production of ridges or furrows, would tend to slow down its speed of travel, from increased friction with the surrounding water. This would have a similar effect to actual fixation in drawing floating particles by the action of the flagellum, and also lead to animal nutrition. Still another development would occur when the fallen flagellate began to creep along the sea-floor by contractile movements of the plasmic surface, losing its flagellum, and adopting the mode of life of an amœba. That amœba and its allies, the Rhizopods, are descended from a flagellate ancestor is a view suggested by Lankester[12] in 1909, which was adopted by Doflein,[13] and is now strongly advocated by Pascher[14] as a result of much new research.

The transformation from the plant to the animal mode of feeding we can see in action by studying actual organisms which exist to-day. In the course of my work already referred to on the culture of plankton organisms there has on several occasions flourished in the flasks a small flagellate belonging to the group of Chrysomonads, which was first described by Wysotzky under the name of *Pedinella hexacostata,* and to which I drew the attention of Section D at the Cardiff Meeting in 1920. The general form of *Pedinella* resembles that of the common Vorticella, but its size is much smaller. The body, which is only about 5 μ in diameter, is shaped like the bowl of a wine glass, and from the base of the bowl, which is the posterior end, a short, stiff stalk extends. From the center of the anterior surface there arises a single long flagellum, surrounded at a little distance by a circle of short, stiff, protoplasmic hairs. Arranged in an equatorial ring just inside the body are six or eight brownish-green chromatophores or chloroplasts. In a healthy cul-

[12] Lankester, "Treatise on Zoology," Part I, London, 1909, p. xxii.

[13] Doflein, "Protozoenkunde," 1916.

[14] Pascher, *Archiv f. Protistenkunde,* Bd. 36, 1916, p. 81, and Bd. 38, 1917, p. 1.

ture *Pedinella* swims about freely by means of a spiral movement of the flagellum, which functions as a tractor, the stalk trailing behind. The chromatophores are large, brightly colored and well developed, and the organism is obviously nourishing itself after the manner of a plant, like any other Chrysomonad. But from time to time a *Pedinella* will suddenly fix itself by the point of the trailing stalk. The immediate effect of this fixing is that a current of water, produced by the still vibrating flagellum, streams towards the anterior surface of the body, and small particles in the water, such as bacteria, become caught up on the anterior surface, the ring of fine stiff hairs surrounding the base of the flagellum being doubtless of great assistance in the capture of this food. One can clearly see bacteria and small fragments of similar size engulfed by the protoplasm of the anterior face of the *Pedinella* and taken into the body. The organism is now feeding as an animal. In some of the cultures in which bacteria were very plentiful nearly all the *Pedinella* remained fixed and fed in the animal way, and when this was so the chromatophores had almost disappeared, though they could still be seen as minute dark dots. We can, as it were, in this one organism see the transition from plant to animal brought about by the simple process of the freely swimming form becoming fixed.

In the group of Dinoflagellates, also—the group to which the naked and armored peridinians belong—the same transition from plant to animal nutrition can be well followed by studying different members of the group. In heavily armored forms, with a rich supply of chromatophores, nutrition is chiefly plant-like or holophytic. In those with fewer chromatophores there is, on the other hand, often distinct evidence of the ingestion of other organisms, and nutrition becomes partly animal-like. Amongst the naked Dinoflagellates such holozoic nutrition is very much developed, and in many species has entirely superseded the earlier method of carbonic acid assimilation.

It is really surprising how many structural features

found in higher groups of animals make their first appearance in these naked Dinoflagellates in conjunction with this change of nutrition, and we seem to be led directly to the metazoa, especially to the Cœlenterata. In *Polykrikos* there are well-developed stinging cells or nematocysts, as elaborately formed as those of Hydra or the anemones. In *Pouchetia* and *Erythropsis* well-developed ocelli are found, consisting of a refractive, hyaline, sometimes spherical lens, surrounded by an inner core of red pigment and an outer layer of black; the whole structure is comparable to the ocelli around the bell of a medusa. In *Noctiluca* and in the allied genus *Pavillardia* a mobile tentacle, which is doubtless used for the capture of food, is developed. Division of the nucleus, with the formation of large, distinct chromosomes, has also been described in several of these Dinoflagellates. With the tendency of the cells in certain species to hold together after division and form definite chains, we seem to approach still nearer to the metazoa, until, finally, in *Polykrikos* we reach an organism which may well have given rise to a simple pelagic cœlenterate. It is difficult to resist the suggestion put forward by Kofoid[15] in his recent monograph, that if to *Polykrikos,* with its continuous longitudinal groove which serves it as a mouth, its multicellular and multinucleate body and its nematocysts, we could add the tentacle of *Noctiluca,* and perhaps also the ocellus of *Erythropsis,* "we should have an organism whose structure would appear prophetic of the Cœlenterata and one whose affinities to that phylum and to the Dinoflagellata would be patent." Or it may be that the older view is the correct one here, and that the first cœlenterate came from a spherical colony of simple holozoic flagellates, arranged something on the plan of *Volvox,* in which the posterior cells of the swimming colony, in whose wake food particles would collect, had become more specialized for nutrition than the rest.

Before proceeding, however, to consider the further

[15] Kofoid and Swezy, "The Free-living Unarmored Dinoflagellata." Mem. Univ. California, 1921.

progress of animal life, we must pause for a moment to ask in what direction plant life in the sea developed, from which the increasing animal life derived its nourishment. Here the striking fact is the lack of progress in the free, floating, plankton phase. The plant life of the plankton has never proceeded beyond the unicellular stage, for the plankton diatoms, which with the peridinians form the great, fundamental vegetable food supply of the sea, are only autotrophic flagellates which have lost their flagella, having acquired other means of flotation to keep them in the sunlit region of the upper water layers. Deriving their food, as these plants do, directly from molecules in the sea-water, the factor which is for them of supreme importance is the exposure of maximum surface directly to the water. Hence the minute unicellular form has been the only one to survive as phytoplankton. The marine region in which plant life has succeeded in making some progress is the narrow belt along the shores, where a fixed life is possible, but this belt, limited by the amount of light which penetrates, extends only to a depth of about 15 fathoms. The available area is further restricted to rocky and hard bottoms, and is therefore nowhere great. This is the wave-lashed region of the brown and red seaweeds. In the brown seaweeds a history can still be traced,[16] from the fixture of an autotrophic flagellate to the building up, by laying cell on cell, of the essential structures which afterwards, on transmigration to the land, reached their climax in the forest tree.

But if the flagellate thus rose and gave origin to the flora of the land, it also degenerated, for it adopted a parasitic habit, living in and directly absorbing already formed organic matter. In this way the bacteria arose, whose activities in so many directions influence the life of to-day. This view exceeds in probability, I think, the suggestion often put forward,[17] that it is to the simpler bacteria we must look for the first beginnings of life.

[16] Church, *Botanical Memoirs*, No. 3. Oxford, 1919.

[17] Osborn, "The Origin and Evolution of Life," 1918. Waksman and Joffe, "Micro-organisms concerned in the Oxidation of Sulphur in the

After this digression on the botanical side we must return to the primitive cœlenterate and see on what lines evolution proceeded in the animal world. As a purely plankton organism, swimming freely in the water, the progress of the cœlenterate was not great, and reached, as far as we know, no further than the modern Ctenophore. The Ctenophore seems to represent the culminating point of the primary progression of pelagic animals, which derived directly from the autotrophic flagellate. Further evolution was associated with an abandonment by a cœlenterate-like animal of the pelagic habit, and the establishment of a connection with the sea bottom, either by fixing to it, by burrowing in it, or by creeping or running over it. At a later stage many of the animals which had become adapted to these modes of life developed new powers of swimming, and thus gave rise to the varied pelagic life which we find in the sea to-day; but this must be regarded as secondary, the primary pelagic life, so far as adult animals were concerned, having ended with the evolution of the Ctenophore.[18] Such is the teaching of embryology, the history of the race being conjectured from the development of the individual. In group after group of the animal kingdom, when the details of its embryology become known, the indications are the same—first the active spermatozoon, reminiscent of the plankton flagellate, then the pelagic larval stage, recalling the cœlenterate, and then a bottom-living phase.

The primitive, free-swimming cœlenterate, adopting a fixed habit and becoming attached mouth upwards to solid rock or stone, gave rise to hydroids, anemones and

Soil,'' *Journal of Bacteriology*, VII, 2, March, 1922. The authors claim that *Thiobacillus thiooxidans* will grow in solutions containing no organic matter. In view of the minute traces of organic matter that suffice for the growth of bacteria and molds, care must be taken, however, in drawing conclusions from experiments made in flasks or tubes closed in the ordinary way with cotton-wool plugs and subsequently sterilized in flowing steam.

[18] There is perhaps a possibility that further knowledge of the embryology of Sagitta and its allies might make it necessary to modify this suggestion.

corals, typical inhabitants of the coastal waters, for the sands and muds at greater depths offered few points of attachment sufficiently stable.

A Volvox-like colony of simple holozoic flagellates, according to MacBride,[19] commenced to feed upon microscopic organisms lying on the sea bottom, and under these circumstances only the cells of the lower half of the colony would be effective feeders. The upper cells, therefore, lost their flagella and became merely a protective layer, which finally grew downwards outside the others and fixed the colony to the ground. In this way a sponge was formed. The collar cell, so typical of the group, had been developed already by the flagellates, its first inception being perhaps a circle of protoplasmic hairs such as we find in *Pedinella*. But this adoption of a fixed habit, as it were mouth downwards, did not lead very far, and though there has been much elaboration within the group itself, the sponges have remained an isolated phylum, unable to develop into higher forms.

It is in a Ctenophore-like ancestor that we find the line of development to higher animal groups, and this ancestor must have been at one time widely distributed in the seas. Its immediate descendants are familiar to every zoological student in the well-known series of pelagic larval forms. Müller's larva, taking to the bottom, and in its hunt for food gliding over hard surfaces with its cilia, led to the flatworms; the *Pilidium*, developing a thread-like body and creeping into cracks and crevices to transfix its prey, gave rise to the nemertines. A Trochophore, burrowing in soft mud and sand, developed a segmented body which gave it later the power of running on these soft surfaces, and became an annelid worm. Another Trochophore, developing a broad, muscular foot, crept on the sand, and afterwards buried itself beneath it as a lamellibranchiate mollusc, or migrated on to harder surfaces as the gastropod and its allies. *Pluteus*, *Bipinnaria*, *Auricularia*, first fixing, as the crinoids still do, and developing a radial symmetry, afterwards broke free and wandered on the bottom as sea-urchin, star-fish

19 "Textbooks of Embryology. Invertebrata." London, 1914.

and cucumarian. *Tornaria* developed into *Balanoglossus,* whose structure hints to us that the ascidians and vertebrates came from a similar stock. All the phyla thus represented derive directly from the free-swimming Ctenophore-like ancestor, and only one considerable group, the Arthropods, remains unaccounted for. The evolutionary history of an Arthropod is, however, not in doubt. Its marine representatives, the Trilobites and Crustacea, came directly from annelids, which, after their desertion of a pelagic life to burrow in the sea-floor and run along its surface, again took to swimming, and not only stocked the whole mass of the water with a rich and varied life of Copepods, Cladocera and Schizopods, but gave rise to Amphipods, Isopods, and Decapods, groups equally at home when roaming on the bottom or swimming above it.

Another important addition to the pelagic fauna we should also notice here. From the molluscs, creeping on solid surfaces, sprang a group of swimmers, the Cephalopods, which have grown to sizes almost unequaled amongst the animals of the sea.

All these invertebrate phyla had become established and most of them had reached a high degree of development in the seas of Cambrian times. Amongst animals then living there are many which have survived with little change of form until to-day. One is almost tempted to suggest that the life which the sea itself could produce was then reaching its summit and becoming stabilized. Since Cambrian times geologists tell us some thirty million years[20] have passed, a stretch of time which it is really difficult for our imaginations to picture. During that time a change of immense moment has happened to the life of the sea; but if we read the signs aright, that change had its origin rather in an invasion from without than in an evolution from within. Whence came that tribe of fishes which now dominates the fauna of the sea? It would be rash to say that we can give any but a speculative reply to the question, but the probable answer seems to be that fishes were first evolved not to meet

[20] Osborn, "Origin and Evolution of Life," 1918, p. 153.

conditions found in the sea, but to battle with the swift currents of rivers, where fishes almost alone of moving animals can to this day maintain themselves and avoid being swept helplessly away.[21] It was in response to these conditions that elongate, soft-bodied creatures, which had penetrated to the river mouth, developed the slender, stream-lined shape, the rigid yet flexible muscular body, the special provision for the supply of oxygen to the blood to maintain an abundant stock of energy, and all those minute perfections for effective swimming that a fish's body shows. The fact that many sea-fishes still return to the rivers, especially for spawning, supports this view, and it is in accordance with Traquair's classical discoveries of the early fishes of the Scottish Old Red Sandstone, which were for the most part fresh- and brackish-water kinds.

Having developed, under the fierce conditions of the river, their speed and strength as swimmers, the fishes returned to the sea, where their new-found powers enabled them to roam over wide areas in search of food, and gave them such an advantage in attack and defense that they became the predominant inhabitants of all the coastal waters, and as such they remain to-day.

The other great migration of the fishes, also, the migration from the water to the land, giving rise to amphibians, reptiles, birds and mammals, must not be left out of account. The whales, seals and sea-birds, which after developing on land returned again to the waters and became readapted for life in them, are features which can not be neglected.

And so we are brought to the picture of life in the sea as we find it to-day. The primary production of organic substance by the utilization of the energy of sunlight in the bodies of minute unicellular plants, floating freely in the water, remains, as it was in the earliest times, the feature of fundamental importance. The conditions which control this production are now, many of them, known. Those of chief importance are (1) the amount of light which

[21] Chamberlin, quoted in Lull, "Organic Evolution," New York, 1917, p. 462.

enters the water, an amount which varies with the length of the day, the altitude of the sun, and the clearness of the air and of the water; (2) the presence in adequate quantity of mineral food substances, especially nitrates and phosphates; and (3) a temperature favorable to the growth of the species which are present in the water at the time. Experiments with cultures of diatoms have shown clearly that if the food-salts required are present, and the conditions as to light and temperature are satisfactory, other factors, such as the salinity of the water and the proportions of its constituent salts, can be varied within very wide limits without checking growth. The increased abundance of plankton, especially of diatom and peridinian plankton, in coastal waters and in shallow seas largely surrounded by land, such as the North Sea, is due to the supply of nutrient salts washed directly from the land by rain or brought down by rivers. An exceptional abundance of plankton in particular localities, which produces an exceptional abundance of all animal life, is also often found where there is an upwelling of water from the bottom layers of the sea. These conditions are met with where a strong current strikes a submerged bank, or where two currents meet. Food-salts which had accumulated in the depths, where they could not be used owing to lack of light, are brought by the upwelling water to the surface and become available for plant growth. The remarkable richness of fish life in such places as the banks of Newfoundland and the Agulhas Banks off the South African coast, each of which is the meeting-place of two great currents, is to be explained in this way.

Our detailed knowledge of the steps in the food-chain from the diatom and peridinian to the fish is increasing rapidly. The Copepod eats the diatom, but not every Copepod eats every diatom; they make their choice. The young fish eats the Copepod, but again there is selection of kind. Even adult fishes like herring and mackerel, which were formerly supposed to swim with open mouth, straining out of the water whatever came in their way, are now thought largely to select their food.[22]

[22] Bullen, *Journ. Mar. Biol. Assoc.*, 9, 1912, p. 394.

A result of extraordinary interest in connection with the food-chain has recently been brought to light by two sets of investigators working independently. In seeking to explain certain features which he had found in connection with the growth of the cod, Hjort[23] undertook a study of the distribution in marine organisms of the growth stimulant known as vitamin. Fat-soluble vitamin was already known to be present in large quantities in cod-liver oil, and is what probably gives the oil its medicinal value. Hjort was able to trace the vitamin, by means of feeding experiments on rats, in the ripe ovaries of the cod, in shrimps and prawns, which resemble the animals on which the cod feeds, and in diatom plankton and green algæ. Jameson, Drummond and Coward[24] cultivated the diatom *Nitzschia closterium*, and by a similar method to that used by Hjort showed that it was extraordinarily potent as a source of fat-soluble vitamin. We thus conclude that this substance, so essential to healthy animal growth, is produced in large quantities by plankton diatoms, and passed on unchanged to the fish through the crustaceans which feed on the diatoms. In the fish the vitamin is first stored in the liver, and with the ripening of the ovary passes into the egg, to be used to stimulate the growth of the next generation. Again we see the fundamental importance of the food-producing activities of the lowest plant life.

Attention has already been drawn to the suggestion that fishes developed their remarkable swimming powers in rivers, in response to a need to overcome the currents, and that they afterwards returned to the sea, where they preyed upon a well-developed and highly complex invertebrate fauna already fully established there. Their speed enabled them to conquer their more sluggish predecessors, whilst they themselves were little open to attack. With the exception of the larger cephalopods, which are of comparatively recent origin, and were probably evolved after the arrival of the fishes, there are few, if any, invertebrates which capture adult fishes as part of their

[23] *Proc. Roy. Soc.*, May 4, 1922.

[24] *Biochemical Journal*, 1922.

normal food. Destructive enemies appeared later in the form of whales and seals and sea-birds, which had developed on the land and in the air.

And now in these last days a new attack is made on the fishes of the sea, for man has entered into the struggle. He came first with a spear in his hand; then, sitting on a rock, he dangled a baited hook, a hook perhaps made from a twig of thorn bush, such as is used to this day in villages on our own east coast. Afterwards, greatly daring, he sat astride a log, with his legs paddled further from the shore, and got more fish. He made nets and surrounded the shoals. Were there time we might trace step by step the evolution of the art of fishing and of the art of seamanship, for the two were bound up together till the day when the trawlers and drifters kept the seas for the battle fleet.

There can be little doubt that in European seas the attack on the fishes in the narrow strip of coastal water where they congregate has become serious. A considerable proportion of the fish population is removed each year, and human activity contributes little or nothing to compensate the loss. We have not, however, to fear the practical extinction of any species of fish, the kind of extinction that has taken place with seals and whales. Fishing is subject to many natural limitations, and when fishing is suspended recovery will be rapid. There is evidence that such recovery took place in the North Sea when fishing was restricted by the War, though the increase which was noted is perhaps not certainly outside the range of natural fluctuations. Until the natural fluctuations in fish population are adequately understood, their limits determined, and the causes which give rise to them discovered, a reliable verdict as to the effect of fishing is difficult to obtain.

If such problems as these are to be solved, the investigation of the sea must proceed on broadly conceived lines, and a comprehensive knowledge must be built up, not only of the natural history of the fishes, but also of the many and varied conditions which influence their lives. The life of the sea must be studied as a whole.

FAMILY RESEMBLANCES AMONG AMERICAN MEN OF SCIENCE

DR. DEAN R. BRIMHALL

SECRETARY OF THE PSYCHOLOGICAL CORPORATION

THE fascinating problems that concern the causes of individual and group differences among human beings are still with us. Since Galton set out to prove that " a man's natural abilities are derived by inheritance under exactly the same limitations as are the form and physical features of the whole organic world " the biological sciences have made many and notable contributions to the fund of knowledge concerning the derivation " of the form and physical features of the organic world." But the influences by which individual and group differences come about, particularly differences in intellectual performance, seem to have been singularly neglected.

The problem has been avoided partly because of the nature of the material. Human beings are not only complicated in organic construction, but they are mongrel in breed, the period between generations is long and direct experimentation impossible. Few scientific problems are more likely to be disturbed by the bias of the experimenter. The American millionaire or European aristocrat explains differences in the wealth gathering or keeping performance of human beings in terms of innate ability. To the socialist the expression " royal minds " has little basis in fact. Laws, taboos, economic and social conditions are thought to be the proper explanations of differences in human behavior. So deeply do the facts concern the fundamental concepts about the organization of society that debate with its anecdotal method should be supplanted by objective method of the best sort available.

The method of approach used in this study is statistical. The investigation represents an attempt to determine some of the differences or resemblances, the causes

of which have been so often the subject of debate and argument. It is necessary to determine what resemblances or differences exist before causes can be explained. This is a study of family resemblances in intellectual performance, particularly in the field of science.

By limiting the problem to the measurement of resemblances the advice of a gifted and successful worker in this field is followed. He writes:

It is impossible at present to estimate with security the relative shares of original nature, due to sex, race, ancestry and accidental variation, and of the environment, physical and social, in causing the differences found in men. One can only learn the facts, interpret them with as little bias as possible, and try to secure more facts.[1]

By this same limitation it is hoped that a serious and common error will be entirely avoided, namely, the failure to realize the twofold nature of the problem of inheritance as ordinarily discussed. The following analysis is so clear and the need of it so general that it is given at length.

Most sociological writers and some biologists are confused in their use of the concept of heredity. When there is discussion of the relative influence on performance of heredity and environment, by heredity there is sometimes understood the original constitution of the individual and sometimes his resemblance to parents and other relatives. It is conceivable that the original constitution of son and father might be exactly the same and yet the individual be so plastic to environment that under different conditions there would be but slight similarity between their performances. It is also conceivable that there might be no similarity between the original constitution of son and father, and yet the performance of each be determined by his original constitution almost without influence from environment. Under which of these extreme hypotheses would the current sociologist call heredity strong or weak? The word heredity should be reserved for resemblance due to a common germ plasm and some other word found for the constitution of the fertilized ovum or zygote; perhaps the best that can be done is to use this uncouth word. We can then discriminate between the two distinct questions: What is the resemblance between the zygotes of two brothers? How far does the zygote of an individual determine his performance as an adult?[2]

[1] Thorndike, "Educational Psychology," Vol. III, p. 310.

[2] J. McKeen Cattell, "Families of American Men of Science," *Pop. Science Monthly*, May, 1915.

It must not be inferred that this study is an attempt to determine "how far the zygote of an individual determines his performance" or "what is the resemblance between the zygotes of two brothers." It is primarily a statistical measurement of resemblance in performance with particular reference to performance in science. With the results of these measurements at hand, something about the resemblances of the zygotes of near relatives and about how far the zygote of an individual determines his performance may be estimated.

By resemblance is meant, not identity, but degree of similarity. Galton, with the idea of particulate inheritance in mind, early insisted on measuring resemblances as deviations from an average. He justly claims to have been the first to "introduce the law of deviations from the average into the discussions on heredity."[3] Almost forty years later he is found insisting that "probability is the foundation of eugenics."[4] It is here that the statistical method avoids the pitfalls of proof by anecdote. Given a group, selected for some particular sort of performance, the number in any particular degree of relationship showing similar performance may be determined and compared with a similar group of the generality. This is the method used in this study.

The group studied consists of approximately 1,000 American men of science and their families. The wives and the near relatives of the wives of the men of science are included in the data, and the results of the comparisons offer perhaps the most unique contribution of the investigation. The statistical data include only relatives of a degree no more remote than first cousin. These relatives are: brothers and sisters, sons and daughters, fathers and mothers, nieces and nephews, uncles and aunts, grandparents and first cousins.

It may be well here to anticipate a criticism of the use of the term resemblance. Since the group of men of science is made up of persons of known distinction and since

[3] "Hereditary Genius," Preface, 1869.

[4] Spencer lecture, 1907.

resemblance of near relatives is measured in terms of the number of the latter who become distinguished, it is assumed that distinction in any intellectual performance is evidence of resemblance. Thus, a psychologist whose cousin is a psychologist may be said without much fear of contradiction to resemble that relative, but does he really resemble his brother, who is a well-known judge? Does he resemble his distinguished father, a former president of the University of Michigan, a gifted administrator?[5] Apparently, that has been assumed. The fact is, they do resemble each other in so far as they vary in the same direction from the average person in the direction of performance.

The selection of the group of scientific men used has become a classic in individual psychology and need not be related in detail here.[6] Briefly, it may be said, the workers in science were grouped in twelve general divisions as follows: Anatomy, anthropology, botany, chemistry, geology, mathematics, pathology, physics, physiology, psychology and zoology. The workers in each science were arranged in an order of merit by ten leading men in each of those sciences. The average position assigned each man, together with the probable error, was computed. Thus each man's position with the reliability of the figure describing his position was determined, and a thousand of the leading men of science were selected as a group for study. Two selections were made, the first in 1903, the second in 1910. The lists varied somewhat in composition due to deaths, changes of position within the group, removal to a foreign country and the like.[7] The positions attained were not published, that

[5] Since the above was written the psychologist, James Rowland Angell, has become a member of the National Academy of Sciences and the president of Yale University. The question becomes less pertinent but the case more dramatic.

[6] J. McKeen Cattell, ''American Men of Science,'' appendix, second edition, 1910.

[7] A third selection has now been made and will furnish material for a continuation of this study. See ''American Men of Science,'' third edition, J. McKeen Cattell and Dean R. Brimhall, 1921.

information being confidential, but the names of those achieving a position in either selection were marked with an asterisk in the handbook in which they were published. They are referred to in this study as the starred group.

The members of the starred group were asked to report among other data the names of relatives as follows:

Relatives who have done scientific work with designation of relationship and direction of work.

Relatives (with designation of relationship and direction of work) sufficient to warrant inclusion in a book such as "Who's Who," say among the first twenty thousand of a hundred million population.

Relatives (with designation of relationship and direction of work) who have done scientific work or work of distinction in other directions.

Professor Cattell, to whom sole credit is due for gathering the original data, writes concerning requests and replies:

> Of one thousand one hundred and fifty-four scientific men from whom information in regard to their families was requested 1,036 replied and 118 did not. Of the replies 16 were blank, sometimes accompanied by the explanation that the information was not readily attainable or the like, 7 were to the effect that the information would be sent later or the like, 13 were received too late, 25 were very imperfect, 975 were usable and in most cases complete. This is an unusually full reply to a questionnaire. For example, in answer to an inquiry in regard to noteworthy relatives addressed to 467 fellows of the Royal Society, Sir Francis Galton received 207 useful replies, and the completely available returns "scarcely exceeded 100." [8]

Following a thorough investigation of that part of the data concerning relatives, in an attempt to supplement and correct them by the use of biographical and genealogical handbooks, the writer sent 186 letters to as many of the men of science, with a report of what had been found in the way of additional information, and asked for corrections and additions. Second and third requests were sometimes sent and in some cases a personal visit to the man of science or near relative was made. As a result the number of usable replies for this study proved to be 956.

[8] "Families of American Men of Science," *Popular Science Monthly*, May, 1915.

Of these 956 records 22 were incomplete in the case of relatives more remote than parents, children, brothers and sisters. Twenty-three were incomplete in kinships more remote than those mentioned. Fully half of the replies of those found to have distinguished relatives were originally incomplete either in names or designation of relationship of names or both. The brother relationship, where it would be supposed complete information would be available, had 84 cases in the original data. This number was raised to nearly 150 through consultation of the handbooks mentioned below. Not more than 25 of those added were found to have first biographical mention at a date later than that of the request for information.

It is unlikely that the ten per cent. who failed to reply did so because of lack of relatives to report; it is unlikely, because that information was a relatively small part of the total requested. Two hundred and fifty-six, or about one fourth of the number replying, were found to have relatives of distinction or relatives who were scientific men; since the other three fourths replied, though they had no relatives to report, it seems reasonable that those who did not reply did not represent a select group. This is further shown to be the case in the number of cross relationships between the two groups. There are found to be brother and cousin relationships that were reported by some of those replying that would have been reported if the others had replied.

The objective criterion used is biographical inclusion in one or more of the three following handbooks: "American Men of Science," "Who's Who in America," "Appleton's Cyclopedia of American Biography." Both editions of "American Men of Science," that is, the editions of 1903 and 1910, were consulted, and biographies found in either were counted. Those found in the original edition of "Appleton's Cyclopedia," published in 1887–88, together with the appendix of 1900 and all ten volumes of "Who's Who in America," covering the pe-

riod from 1910 to 1918, were also included. The first edition of "American Men of Science" contains more than 4,000 men of science, of entire North America, the second edition about 5,500 names. "Appleton's Cyclopedia of American Biography (1887–88)" contains "above 15,000 prominent native and adopted citizens of the United States, including living persons, from the earliest settlement of the country." In the appendix of 1899–00 "will be found nearly 2,000 notices of Americans who won renown in the war with Spain . . . and of persons of the New World who have become prominent in the peaceful activities of life during the decade," between the appearance of the two publications. The ten volumes of "Who's Who in America" contain 36,915 biographical sketches. The first volume contains 8,602 biographies, while Volume 10 has 22,968. It is evident that the three publications have varying standards of selection, and it becomes necessary to get some statement of the degree of fineness of selection represented by each.

If the reader doubts the validity of any one of the three measures he may disregard those found in that handbook because the lists of names and tables are arranged to that end. That there are biographies of persons included that are out of place is likely and that omissions of others quite deserving occur is also likely, but inclusion represents unusual performance that is a reality.

There is given below the biographical account of one of the persons in the study as it is given in the three different handbooks. Besides adding reality to the data in the lists it will afford a comparison of the characteristic methods employed by the editors of the different publications. The accounts give some idea of the interesting and voluminous records that would be necessary if no more than a brief history of each individual were given. The histories of the men of science and their relatives, if abbreviated in the most careful manner, would make a fair-sized volume. One need only imagine the size of the volume necessary to give an account of the unusual per-

formance of an equal number of people taken at random to see the difference.

Biographical Account of Edward Charles Pickering, American Men of Science, 1910

Pickering, Prof. Edward C(harles), Harvard College Observatory, Cambridge, Mass. * *Astronomy, Astrophysics.* Boston, Mass, July 19, 46. B.S, Harvard, 65, A.M, 80, LL.D, 03; California, 86; Michigan, 87; Sc.D, Victoria (England), 00; LL.D, Chicago, 01; Ph.D, Heidelberg, 03; LL.D, Pennsylvania, 06. Instr. math, Lawrence Sci. Sch, Harvard, 65–67; *prof.* physics, Mass. Inst. Tech, 67–77; *astron. and director, Harvard Col. Observatory,* 77– Bruce Gold Medal, Pacific Astron. Soc; Rumford, Draper, Bruce, two Royal Astron. Soc. and other medals. Nat. Acad; F.A.A. (v. pres, 77); Astron. and Astrophys. Soc. (pres, 06–08); Philos. Soc. (v. pres, 09); fel. Am. Acad; Wash. Acad; hon. mem. N. Y. Acad; cor. mem. Berlin Acad; Soc. astron. de France; Inst. de France; Royal Soc. Upsala; Soc. Lynceorum Nova; St. Petersburgh Imp. Acad; Societies of Cherbourg, Palermo, etc. Stellar photometry and spectroscopy.

Account given in Who's Who in America, Vol. 6, 1910–11

Pickering, Edward Charles, astronomer; *b.* Boston, July 19, 1846. *s.* Edward and Charlotte (Hammond) P; brother of William Henry P. (*q.v.*); ed. Boston Latin School; S.B, Lawrence Scientific Sch. (Harvard), 1865 (hon. A.M., 1880; LL.D., univs. of Cal., 1886, Mich., 1887, Chicago, 1901, Harvard, 1903, Pa., 1906; Ph.D., Heidelberg, 1903; D.Sc., Victoria U., Eng., 1900; *m.* Lizzie Wadsworth, *d.* Jared Sparks, Mar. 9, 1874. Instr. mathematics, Lawrence Scientific Sch., 1865–7; Thayer prof. physics, Mass. Inst. Tech., 1867–76; prof. astronomy and dir. Harvard Coll. Obs. since 1876. Established 1st physical lab. in U. S.; under his direction, invested capital and income of the observatory has increased fourfold. Study of light and spectra of the stars have been spl. features of his work; devised meridian photometer and made 1,400,000 measures of the light of the stars with it. By establishing an auxiliary sta. in Arequipa, Peru, Southern stars are also observed, extending the work from pole to pole, in which 200,000 photographs are included. Accompanied Nautical Almanac expdn. to observe total eclipse of sun, Aug. 7, 1869; mem. U. S. Coast Survey expdn. to Xeres, Spain, Dec. 22, 1870. Awarded Henry Draper medal for work on astron. physics; gold medals, Rumford, 1891, Bruce, 1908, Royal Astron. Soc., 1886, 1901. Mem. Nat. Acad. Sciences; hon. mem. of Socs. at Mexico, Cherbourg, Liverpool, Toronto, Upsala and Lund; mem. Royal Astron. Soc., Royal Instn. Acaad. dei Lincei, Royal Prussian, and Royal Irish socs., Royal Soc. of London, Institute de France, Imperial Acad., St. Petersburg; pres. Astron. and Astrophys. Soc.

America, 1906–9; fellow Am. Acad. Arts and Sciences; founder and 1st pres. Appalachian Mountain Club; mem. Century Assn., New York. *Author:* Elements of Physical Manipulation, and 60 volumes of annals and other publications of Harvard Coll. Observatory. *Address:* Harvard Observatory, Cambridge, Mass.

Account given in Appleton's Cyclopedia of American Biography, 1887–8

Pickering, Edward Charles, astronomer, b. in Boston, Mass., 19 July, 1846, was graduated in civil engineering course at the Lawrence scientific school of Harvard in 1865. During the following year he was called to the Massachusetts institute of technology as assistant director of physics, of which branch he held the full professorship from 1868 till 1877. Prof. Pickering devised plans for the physical laboratory of the institution, and introduced the experimental method of teaching physics at a time when that mode of instruction had not been adopted elsewhere. His scientific work of these years consisted largely of researches in physics, notably investigations on the polarization of light and the laws of its reflection and dispersion. He also described a new form of spectrum telescope, and invented in 1870 a telephone receiver, which he publicly exhibited. He observed the total eclipse of the sun on 7 Aug., 1869, with the party that was sent out by the Nautical almanac office, at Mt. Pleasant, Iowa, and was a member of the U. S. coast survey expedition to Xeres, Spain, to observe that of 22 Dec., 1870, having, on that occasion, charge of the polariscope. In 1876 he was appointed professor of astronomy and geodesy, and director of the observatory at Harvard, and under his management this observatory has become one of the foremost in the United States. More than twenty assistants now take part in investigations under his direction and the invested funds of the observatory have increased from $176,000 to $654,000 during his administration. His principal work since he accepted this appointment has been the determination of the relative brightness of the stars, which is accomplished by the means of a meridian photometer, an instrument specially devised for this purpose, and he has prepared a catalog giving the brightness of over 4,000 stars. Since 1878 he has also made photometer measurements of Jupiter's satellites while they are undergoing eclipse, and of the satellites of Mars and other faint objects. On the death of Henry Draper (q.v.) his widow requested Prof. Pickering to continue important researches on the application of photography to astronomy, as a Henry Draper memorial, and the study of the spectra of the stars has been undertaken on a scale that was never before attempted. A fund of $250,000, left by Uriah A. Boyden (q.v.) to the observatory, has been utilized for the special study of the advantages of very elevated observing stations. Prof. Pickering has also devoted attention to such objects as mountain surveying, the height and velocity of clouds, papers on which he

has contributed to the Appalachian Club, of which he was president in 1877, and again in 1882. He is an associate of the Royal astronomical society of London, from which in 1886 he received its gold medal for photometric researches, and besides membership in other scientific societies in the United States and Europe he was elected in 1873 to the National academy of sciences, by which body he was further honored in 1887 with the award of the Henry Draper medal for his work on astronomical physics. In 1876 he was elected a vice-president of the American association for the advancement of science, and presented his retiring address before the section of mathematics and physics at the Nashville meeting. In addition to his many papers which number about 100, he prepared "Reports on the Department of Physics," for the Massachusetts institute of technology, and the "Annual Reports of the Director of the Astronomical Observatory," likewise editing the "Annals of the Astronomical Observatory of Harvard College." He has also edited with notes "The Theory of Color in Relation to Art and Art Industry," by Dr. William von Bezold (Boston, 1876), and he is the author of "Elements of Physical Manipulation" (2 parts, Boston, 1873–6).

The raw material of the investigation is found below in the lists of men of science and their relatives. Statistical treatment of these data will follow. They are arranged so that any competent observer can test their validity. While great effort has been made to have the details reliable, it is possible that mistakes may be found in the designation of relationship, but it is thought that such mistakes are few if any.

The Men of Science and their Near Relatives of Distinction

The names of the men of science and their near relatives of distinction are given at the left of the page. The name of the man of science comes first and is followed by a short dash. The names of the relatives follow and are preceded by the letter or letters which tell the relationship. The first name, for example, is Allis, Edward Phelps; he has a cousin (FSiS, a father's sister's son), Callahan, Henry White, whose work is in education. The biography of this relative is found in "Who's Who in America." When the name of a relative is printed in small capitals it shows that the person is known for work

in science. The direction of the performance of the relatives is given at the right of the name. The handbook or books containing his biographical account are shown by the abbreviations at the right. These abbreviations are to be understood as follows: A.M.S, American Men of Science (if preceded by an asterisk the person is in the starred group of men of science); W.W, Who's Who in America; A.C, Appleton's Cyclopedia of American Biography.

Any degree of relationship can be conveniently and accurately described by one, or a combination of two or more, of the following seven letters: S for son, D for daughter, B for brother, Si for sister, F for father, M for mother. Thus, paternal grandfather can be written as FF, meaning father's father, maternal grandfather, MF, meaning mother's father, FBS meaning first cousin or father's brother's son, etc. These symbols precede the name and show the kinship of the person to the man of science.

The men of science are arranged according to the science in which they were recorded as obtaining a place in the starred group. The groups are arranged alphabetically, beginning with the anatomists and closing with the zoologists.

Starred anatomists and near relatives of distinction		Direction of performance	Handbooks containing biographical accounts of relatives
	Anatomists		
Allis, Edward Phelps—			
FSiS	Callahan, Henry White	Education	W.W.
Bardeen, Charles Russell—			
F	Bardeen, Charles William	Education	W.W.
Bensley, Robert Russell—			
B	Bensley, Benjamin Arthur	Zoology	A.M.S.
Dwight, Thomas—			
MB	Warren, Jonathan Mason	Surgery	A.C.
MF	Warren, John Collins	Surgery	A.C.
MBS	Warren, John Collins	Surgery	*A.M.S; W.W: A.C.
FBS	Dwight, Wilder	Warfare	A.C.
FBS	Dwight, William	Warfare	W.W.

Greenman, Milton Jay—			
FBS	GREENMAN, JESSE MORE	Botany	*A.M.S; W.W.
Meyer, Arthur William—			
B	Meyer, Balthassar Henry	Polit. econ.	W.W.
Spitzka, Edward Anthony—			
F	SPITZKA, EDWARD A.	Neurology	A.M.S; A.C.

Anthropologists

Dorsey, George Amos—			
B	DORSEY, CLARENCE W.	Soil physics	W.W.
B	DORSEY, HERBERT GROVE	Physics	A.M.S.
Farrand, Livingston—			
B	Farrand, Max	History	W.W.
B	Farrand, Wilson	Education	W.W.
Hough, Walter—			
FBS	HOUGH, THEODORE	Physiology	*A.M.S; W.W.

Astronomers

Doolittle, Charles Leander—			
S	DOOLITTLE, ERIC	Astronomy	*A.M.S; W.W.
Doolittle, Eric—			
F	DOOLITTLE, CHARLES L.	Astronomy	*A.M.S; W.W.
Frost, Edwin Brant—			
B	FROST, GILMAN DUBOIS	Anatomy	A.M.S.
Lowell, Percival—			
B	Lowell, Abbott Lawrence	Education	W.W.
Si	Lowell, Amy	Poetry	W.W.
MF	Lawrence, Abbot	Diplomacy	A.C.
FSiD	Cabot, Ella Lowell Lyman	Education	W.W.
MSiS	ROTCH, ABBOTT LAWRENCE	Physics	*A.M.S; W.W.
MSiS	Rotch, Arthur	Architecture	A.C.
Pickering. Edward Charles—			
B	PICKERING, WILLIAM H.	Astronomy	*A.M.S; W.W; A.C.
FB	PICKERING, CHARLES	Ethnol; Bot.	A.C.
Pickering, William Henry—			
B	PICKERING, EDWARD C.	Astronomy	*A.M.S.
FB	PICKERING, CHARLES	Ethnol; Bot.	A.C.
Pritchett, Henry Smith—			
F	Pritchett, Car Waller	Ministry; Educ.	W.W.

Searle, Arthur—		Astron; Religion	A.M.S; W.W; A.C.
B	SEARLE, GEORGE MARY		
Stone, Ormond—		Journ; Finance	
B	Stone, Melville Elijah		W.W; A.C.
Wright, William Hammond—			
M	Wright, Johanna Maynard	Organization	W.W.

Botanists

Beal, William James—			
MSiS	STEERS, JOSEPH BEAL	Zoology	A.M.S; W.W.
Bessey, Ernst Athern—			
F	BESSEY, CHARLES EDWIN	Botany	*A.M.S; W.W.
Bessey, Charles Edwin—			
S	BESSEY, ERNST ATHERN	Botany	*A.M.S; W.W.
Blakeslee, Albert Francis—			
B	Blakeslee, George Hubbard	History	W.W.
F	Blakeslee, Francis Durbin	Ministry	W.W.
Bray, William—			
MBS or FSiS	FOSTER, LUTHER	Botany	W.W.
Campbell, Douglas Houghton—			
B	CAMPBELL, EDWARD DEM.	Chemistry	*A.M.S; W.W.
B	Campbell, Henry Munroe	Law	W.W.
F	Campbell, James V.	Law	A.C.
Coker, William Chambers—			
F	Coker, James Lide	Manufacturing	W.W.
FBS	COKER, ROBERT IRWIN	Zoology	A.M.S; W.W.
Coulter, John Merle—			
B	COULTER, STANLEY	Botany	*A.M.S; W.W.
S	COULTER, JOHN GAYLORD	Botany	A.M.S.
FBS	COULTER, SAMUEL MONDS	Botany	A.M.S.
Coulter, Stanley—			
B	COULTER, JOHN MERLE	Botany	*A.M.S; W.W; A.C.
BS	COULTER, JOHN GAYLORD	Botany	A.M.S.
FBS	COULTER, SAMUEL MONDS	Botany	A.M.S.
Coville, Fred Vernon—			
B	COVILLE, LUZERNE	Medicine	A.M.S.
Davis, Bradley Moore—			
FSiS	WOOD, ROBERT WILLIAMS	Physics	*A.M.S; W.W.

Duggar, Benjamin Minge—			
B	DUGGAR, JOHN FREDERIC	Agronomy	A.M.S; W.W.
Earle, Franklin Sumner—			
Si	Horne, Mary Tracy Earle	Fiction	W.W.
F	EARLE, PARKER	Horticulture	A.C.
Fernald, Merritt Lyndon—			
B	FERNALD, ROBERT H.	Mech. eng.	A.M.S; W.W.
F	FERNALD, MERRITT C.	Educ; Physics; Math.	A.M.S; W.W.
Greenman, Jesse More—			
FBS	GREENMAN, MILTON JAY	Anatomy	*A.M.S; W.W.
Halstead, Byron David—			
SiS	FAIRCHILD, DAVID G.	Botany	*A.M.S; W.W.
SiS	Fairchild, Edwin Milton	Education	W.W.
Kearney, Thomas H.—			
MB	Miner, Charles Wright	Warfare	W.W.
Macbride, Thomas Huston—			
FSiS	Sterrett, James M.	Ministry; Hist.	W.W.
Pinchot, Gifford—			
B	Pinchot, Amos	Law; Politics	W.W.
F	Pinchot, James W.	Trade	W.W.
MF	Eno, Amos R.	Finance	A.C.
Pound, Roscoe—			
Si	Pound, Louise	Philology	W.W.
Robinson, Benjamin Lincoln—			
B	Robinson, James H.	History	W.W.
Shull, George Harrison—			
B	SHULL, CHARLES ALBERT	Zoology	A.M.S.
B	Scholl, John W.	Literature	W.W.

Chemists

Acheson, Edward Goodrich—			
FB	Acheson, Marcus Wilson	Law	W.W.
FBS	Acheson, Alexander M.	Civil Eng.	W.W.
FBS	Acheson, Alexander W.	Med; Politics	W.W.
FBS	Acheson, Ernest Francis	Journal; Politics	W.W.
FBS	Acheson, Marcus W. Jr.	Law	W.W.
FSiS	Brownson, Marcus A.	Ministry	W.W.
FSiD	Brownson, Mary Wilson	Literature; Math.	W.W.
Bancroft, Wilder Dwight—			
FF	Bancroft, George	History	A.C.

Blair, Andrew Alexander—			
F	Blair, Francis Preston	Warfare; Politics	A.C.
FF	Blair, Francis Preston	Journal; Politics	A.C.
Burgess, Charles Frederick—			
B	Burgess, George H.	Railway Eng.	W.W.
Campbell, Edward DeMille—			
B	CAMPBELL, DOUGLAS H.	Botany	*A.M.S; W.W.
B	Campbell, Henry Munroe	Law	W.W.
F	Campbell, James V.	Law	A.C.
Chatard, Thomas Marean—			
B	Chatard, Francis Silas	Educ; Ministry; Med.	W.W; A.C.
FB	Chatard, Frederick	Warfare	A.C.
Crafts, James Mason—			
MF	Mason, Jeremiah	Law	A.C.
Dabney, Charles William—			
F	Dabney, Robert Lewis	Educ; Ministry	A.C.
Doremus, Charles Avery—			
F	DOREMUS, ROBERT O.	Chemistry	A.M.S; W.W; A.C.
FM	Doremus, S. P. (Haines)	Philanthropy	A.C.
Dunnington, Francis Perry—			
MB	Keener, John C.	Ministry	W.W; A.C.
Franklin, Edward Curtis—			
B	FRANKLIN, WILLIAM S.	Physics	*A.M.S; W.W.
Freer, Paul Casper—			
B	Freer, Frederick W.	Art (Painting)	W.W.
B	FREER, OTTO TIGER	Laryngology	W.W.
Hilgard, Eugene Woldemar—			
B	HILGARD, JULIUS	Math; Geodesy	A.C.
B	HILGARD, THEODORE C.	Biology	A.C.
F	HILGARD, THEODORE E.	Law	A.C.
SiS	TITTMAN, OTTO HILGARD	Geodesy	A.M.S; W.W.
Jackson, Charles Loring—			
FSi	Lowell, Anna C. J.	Education	A.C.
FF	Jackson, Patrick Tracy	Manufacturing	A.C.
MF	Loring, Charles Greely	Insurance; Law	W.W; A.C.
FSiS	CABOT, ARTHUR TRACY	Surgery	W.W.
MBS	Loring, William Caleb	Law	W.W.
FSiS	Lowell, C. R.	Warfare	A.C.
Lewis, Gilbert Newton—			
FB	Lewis, Homer Pierce	Education	W.W.

Lloyd, John Uri—			
B	LLOYD, CURTIS GATES	Botany	A.C.
Loeb, Morris—			
B	Loeb, James	Finance; Archeol.	W.W.
More, Richard Bishop—			
F	Moore, William Thomas	Ministry; Editing	W.W.
MF	Bishop, Richard Moore	Politics; Trade	A.C.
Morely, Edward Williams—			
B	Moreley, John Henry	Ministry; Educ.	W.W.
Munroe, Charles Edward—			
FBS	Munroe, James Phinney	Mfg; Writing	W.W.
FBS	Munroe, Kirk	Fiction; Journal	W.W.
Norris, James Flack—			
B	NORRIS, RICHARD C.	Surgery	W.W.
Norton, Thomas Herbert—			
MB	HORSFORD, EBEN NORTON	Chemistry	A.C.
MF	Horsford, Jerediah	Warfare	A.C.
MBD	HORSFORD, CORNELIA	Archeology	W.W.
FBS	NORTON, LEWIS MILLS	Chemistry	A.C.
Noyes, William Albert—			
Si	Davidson, Hanna M. N.	Education; Lit.	A.C.
Orndorf, William Ridgely—			
MF	Ridgely, James Lot	Law	A.C.
Osborne, Thomas Burr—			
MB	BLAKE, ELI WHITNEY	Chem; Physics	A.C.
MF	Blake, Eli Whitney	Invention; Mfg.	A.C.
Palmer, Chase—			
FSiS	Harris, George	Ministry; Lit.	W.W.
MBS	Chase, George	Educ; Law	W.W.
Pellew, Charles Ernest—			
F	Pellew, Henry Edward	Philanthropy	W.W.
MB	Jay, John	Diplomacy	A.C.
MF	Jay, William	Law	A.C.
MBS	Jay, William	Law	W.W.
Pond, George Gilbert—			
½B	POND, FRANCIS JONES	Chemistry	A.M.S.
Reese, Charles Lee—			
B	Reese, Frederick Focke	Ministry	W.W.
Richards, Theodore William—			
B	RICHARDS, HERBERT M.	Botany	*A.M.S; W.W.
F	Richards, William Trost	Art (Painting)	W.W; A.C.

Sadtler, Samuel Philip—			
F	Sadtler, Benjamin	Ministry	A.C.
MB	Schmucker, B. Melanchton	Ministry	A.C.
MB	Schmucker, Samuel M.	Writing	A.C.
MB	Schmucker, Samuel D.	Law	W.W.
MF	Schmucker, Samuel S.	Educ; Theol.	A.C.
MBS	SCHMUCKER, SAMUEL C.	Botany	A.M.S; W.W.
Sanger, Charles Robert—			
F	Sanger, George P.	Law	A.C.
Saunders, Arthur Percy—			
B	SAUNDERS, CHARLES E.	Chemistry	A.M.S.
B	SAUNDERS, FREDERICK A.	Physics	*A.M.S; W.W.
B	SAUNDERS, WILLIAM E.	Ornith; Bot.	A.M.S.
F	SAUNDERS, WILLIAM	Horticulture	A.M.S.
Sherman, Henry Clapp—			
B	SHERMAN, FRANKLIN, JR.,	Entomology	A.M.S.
FBS	Sherman, Frank D.	Architect; Poetry	W.W.
Shimer, Porter William—			
FBS	SHIMER, HERVEY W.	Geology	A.M.S; W.W.
Smith, Edgar Fahs—			
B	SMITH, ALLEN JOHN	Pathology	A.M.S; W.W.
Stieglitz, Julius Oscar—			
B	Stieglitz, Alfred	Photog; Chem.	W.W.
Stillman, John Maxon—			
½B	STILLMAN, STANLEY	Surgery	W.W.
FB	Stillman, Thomas Bliss	Manufacturing	A.C.
FB	Stillman, William James	History; Journal	W.W; A.C.
FBS	STILLMAN, THOMAS B.	Chemistry	*A.M.S; W.W.
Stillman, Thomas Bliss—			
FB	Stillman, Thomas Bliss	Manufacturing	A.C.
FB	Stillman, William James	History; Journal	W.W; A.C.
FBS	STILLMAN, JOHN MAXON	Chemistry	*A.M.S; W.W.
FBS	STILLMAN, STANLEY	Surgery	W.W.
Tuckerman, Alfred—			
B	Tuckerman, Bayard	History	W.W.
MB	Gibbs, Alfred	Warfare	A.C.
MB	Gibbs, George	Antiquarianism	A.C.
MB	GIBBS, OLIVER WOLCOTT	Chemistry	*A.M.S; W.W; A.C.
FF	Tuckerman, Joseph	Ministry	A.C.
MF	GIBBS, GEORGE	Geology	A.C.
FSiS	BECKER, GEORGE F.	Geology	*A.M.S; W.W.
VanSlyke, Lucius Lincoln—			
S	VANSLYKE, DONALD D.	Chemistry	A.M.S.

Venable, Francis Preston—			
F	VENABLE, CHARLES S.	Astronomy	W.W; A.C.
MF	McDowell, James	Politics	A.C.
Waller, Elwyn—			
B	Waller, Frank	Architecture	W.W; A.C.

Geologists

Ashley, George Hall—			
B	Ashley, Roscoe Lewis	History; Econ.	W.W.
Becker, George Ferdinand—		Ministry	A.C.
MF	Tuckerman, Joseph	Chemistry	*A.M.S; W.W.
MBS	TUCKERMAN, ALFRED		
MSiS	Tuckerman, Bayard	Biography; Hist.	W.W.
Brooks, Alfred Hulse—			
F	BROOKS, THOMAS B.	Geology	A.C.
Si	Paige, Hildegard B.	Fiction	W.W.
Chamberlin, Thomas Corwin—			
S	CHAMBERLIN, ROLLIN T.	Geology	A.M.S; W.W.
Clarke, John Mason—			
B	Clarke, Lorenzo Mason	Ministry	W.W.
Dana, Edward Salisbury—			
F	DANA, JAMES DWIGHT	Geology	A.C.
MB	SILLIMAN, BENJAMIN JR.	Chemistry	A.C.
MF	SILLIMAN, BENJAMIN	Chem; Geol.	A.C.
Davis, William Morris—		Philanthropy	A.C.
MF	Mott, James	Ministry	
MM	Mott, Lucretia	(Quaker)	A.C.
Farrington, Oliver Cummings—		Journalism;	W.W.
B	Farrington, Wallace R.	Hist.	
B	FARRINGTON, EDWARD H.	Chemistry	W.W.
Gilbert, Grove Karl—			
F	Gilbert, Grove Sheldon	Art	A.C.
Grant, Ulysses Sherman—			
F	Grant, Lewis Addison	Law; Warfare	W.W; A.C.
Hague, Arnold—			
B	HAGUE, JAMES DUNCAN	Geology	A.M.S; W.W; A.C.
F	Hague, William	Ministry	A.C.
Harris, Gilbert Dennison—			
B	HARRIS, ROLLIN ARTHUR	Geodesy	A.M.S; W.W.

Hayes, Charles Willard—			
Si	HAYES, ELLEN	Mathematics	A.M.S.
Hitchcock, Charles Henry—			
F	HITCHCOCK, EDWARD	Geol; Educ.	A.C.
B	HITCHCOCK, EDWARD	Hygiene	A.M.S; W.W; A.C.
BS	HITCHCOCK, EDWARD, JR.	Hygiene	A.M.S; W.W.
Irving, John Duer—			
F	IRVING, ROLAND DUER	Geology	A.C.
Jaggar, Thomas Augustus, Jr.—			
F	Jaggar, Thomas A.	Ministry	W.W; A.C.
Keith, Arthur—			
MSiS	GALE, HOYT STODDARD	Geology	A.M.S.
Mathews, Edward Bennett—			
B	Mathews, Shailer	Educ; Theol.	W.W.
Merriam, John Campbell—			
B	Merriam, Charles E.	Politics; Hist.	W.W.
Merrill, George Perkins—			
B	MERRILL, LUCIUS H.	Chemistry	A.M.S; W.W.
Penrose, Richard Alex. Fullerton—			
B	Penrose, Boies	Politics; Law	W.W.
B	PENROSE, CHARLES B.	Surg; Physics	A.M.S; W.W.
B	Penrose, Spencer	Eng; Finance	W.W.
F	PENROSE, RICHARD A. F.	Surgery	A.M.S; W.W; A.C.
FB	Penrose, Clement B.	Law	W.W.
FF	Penrose, Charles B.	Law	A.C.
FBS	Penrose, Stephen B. L.	Educ; Philos.	W.W.
Pumpelly, Raphael—			
D	Smyth, Margarette P.	Art (Painting)	W.W.
M	Pumpelly, Mary Weller	Poetry	A.C.
Rice, William North—			
S	RICE, EDWARD LORANUS	Zoology	*A.M.S; W.W.
Scott, William Berryman—			
B	SCOTT, HUGH LENNOX	Warfare; Anthrop.	W.W.
MB	Hodge, Archibald Alex.	Theology	A.C.
MF	Hodge, Charles	Theology	A.C.
Smith, James Perrin—			
B	Smith, Charles Forster	Philology	W.W.
Stevenson, John James—			
MB	Willson, James McLeod	Philol; Theol.	A.C.
MF	Willson, James Renwick	Philol; Theol.	A.C.
MBS	Willson, David Burt	Philol; Theol.	W.W; A.C.

Taylor, Frank Bensley—			
F	Stewart, Robert	Politics; Law	W.W.
Vaughn, Thomas Wayland—			
FBS	Vaughn, Horace Worth	Politics; Law	W.W.
Weed, Walter Harvey—			
F	Weed, Samuel Richards	Finance; Lit.	W.W.
Weller, Stewart—			
MB	Marran, J. T.	Law	W.W.
White, David—			
MSiS	Kent, Charles Foster	Hist; Archeol.	W.W.
Willis, Bailey—			
F	Willis, Nathaniel P.	Poetry	A.C.
FB	Willis, Richard Storrs	Music	A.C.
Winchell, Alexander Newton—			
B	WINCHELL, HORACE V.	Geology	A.M.S; W.W.
F	WINCHELL, NEWTON H.	Geol; Archeol.	*A.M.S; W.W; A.C.
FB	WINCHELL, ALEXANDER	Geology	A.C.
FB	Winchell, Samuel R.	Educ; Journalism	W.W.
Winchell, Newton Horace—			
B	WINCHELL, ALEXANDER	Geology	A.C.
B	Winchell, Samuel R.	Educ; Journalism	W.W.
S	WINCHELL, ALEX. N.	Geology	*A.M.S; W.W.
S	WINCHELL, HORACE V.	Geology	A.M.S; W.W.
FBS	Winchell, Benj. La Fon	Ry. Management	W.W.
FB	Winchell, James M.	Ministry	A.C.
Wright, Frederic Eugene—			
B	WRIGHT, CHARLES WILL	Geology	A.M.S.

Mathematicians

Birkoff, George David—			
MB	Droppers, Garrett	Polit. Econ.	W.W.
Coolidge, Julian Lowell—			
B	Coolidge, Archibald C.	History	W.W.
B	Coolidge, John Gardiner	Diplomacy	W.W.
B	Coolidge, J. Randolph Jr.	Architecture	W.W.
FB	Coolidge, Jefferson	Diplomacy	W.W; A.C.
FBS	Coolidge, T. Jefferson Jr.	Finance	W.W.
Fine, Henry Burchard—			
FF	Fine, John	Politics; Law	A.C.

Franklin, Fabian—			
MB	Heilprin, Michael	History; Sociol.	A.C.
MF	Heilprin, Phineas M.	Semitics	A.C.
MBS	HEILPRIN, ANGELO	Geology	*A.M.S; W.W; A.C.
MBS	Heilprin, Louis	Philology	W.W; A.C.
Halsted, George Bruce—			
F	Halsted, Oliver Spencer	Politics	A.C.
FF	Halsted, Oliver Spencer	Philology	A.C.
Johnson, William Woolsey—			
B	Johnson, Charles Fred.	Philology; Math.	W.W.
McClintock, Emory—			
F	McClintock, John	Educ; Ministry	A.C.
Moore, Eliakim Hastings—			
F	Moore, David Hastings	Educ; Ministry	W.W.
Pierce, Charles Santiago Sanders—			
B	Peirce, Herbert H. D.	Diplomacy	W.W.
B	PEIRCE, JAMES MILLS	Math; Educ.	*A.M.S; W.W; A.C.
F	PEIRCE, BENJAMIN	Mathematics	A.C.
FB	PEIRCE, CHARLES HENRY	Chem; Med.	A.C.
FF	Peirce, Benjamin	Library (Harvard)	A.C.
MF	Mills, Elijah Hunt	Politics	A.C.
Roe, Edward Drake Jr—			
FB	Roe, Francis Asbury	Warfare	W.W; A.C.
Slichter, Charles Summer—			
BS	SLICHTER, WALTER I.	Elec. Eng.	A.M.S; W.W.
Van Vleck, Edward Burr—			
F	VAN VLECK, JOHN M.	Astron; Math.	A.M.S; W.W; A.C.
Veblen, Oswald—			
F	VEBLEN, ANDREW A.	Math; Physics	A.M.S; W.W.
FB	Veblen, Thorstein B.	Economics	W.W.
Wilson, Edwin Bidwell—			
FB	Wilson, Frank E.	Politics; Law	W.W.

Pathologists

Biggs, Herman Michael—			
FBS	BIGGS, GEORGE PATTEN	Pathology	A.M.S.
Blumer, George—			
FBS	BLUMER, GEORGE ALDER	Neurol; Med.	W.W.

Cabot, Richard Clarke—			
FBS	CABOT, ARTHUR TRACY	Med; Surg.	W.W.
FBS	CABOT, GODFREY L.	Chemistry	W.W.
Christian, Henry Asbury—			
FBS	Christian, George L.	Politics; Lit; War	W.W.
Cushing, Harvey Williams—			
B	CUSHING, HENRY	Geology	A.M.S; W.W.
B	Cushing, William E.	Law	W.W.
MSiS	CREHORE, ALBERT C.	Physics; Eng.	*A.M.S; W.W.
MSiS	CREHORE, WILLIAM W.	Mech. Eng.	W.W.
Dana, Charles Loomis—			
B	Dana, John Cotton	Library	W.W.
Dock, George—			
Si	DOCK, LAVINA L.	Medicine	W.W.
Ernst, Harold Clarence—			
B	Ernest, George Alex.	Law	W.W.
B	ERNST, OSWALD H.	Astron; War; Eng.	W.W.
MF	OTIS, GEORGE ALEX.	Surgery	A.C.
Flexner, Simon—			
B	Flexner, Abraham	Education	W.W.
Hurd, Henry Mills—			
½B	HURD, ARTHUR W.	Psychiatry	W.W.
Loeb, Leo—			
B	LOEB, JACQUES	Zoology	*A.M.S; W.W.
MacCallum, William George—			
B	MACCALLUM, JOHN B.	Anatomy	*A.M.S; W.W.
F	MACCALLUM, GEORGE A.	Ornith; Med.	W.W.
Mitchell, Silas Weir—			
S	MITCHELL, JOHN K.	Neurology	A.M.S; W.W.
S	Mitchell, Langdon E.	Playwriting	W.W.
F	MITCHELL, JOHN K.	Chem; Med.	A.C.
Musser, John Herr—			
MBS or FSiS	Herr, Edwin M.	Elec. Eng.	W.W.
Park, William Hallock—			
MF	Hallock, William S.	Editing; Ministry	A.C.
MSiS	Johnson, William H.	Ministry; Philos.	W.W.
Putnam, James J.—			
MF	Jackson, James	Clin. Med.	A.C.

Ravenel, Mazyck Porcher—

FBS	Ravenel, Henry W.	Botany	A.C.
MBS	Porcher, Francis P.	Botany; Chem.	A.C.

Thayer, William Sydney—

B	Thayer, Ezra Ripley	Law	W.W.
F	Thayer, James Bradley	Law	A.C.
MSiS	Simons, Edward	Art (Painting)	W.W.

Warren, John Collins—

F	Warren, Jonathan M.	Surg; Med.	A.C.
FF	Warren, John Collins	Surgery	A.C.
FSiS	Dwight, Thomas	Anatomy	*A.M.S; W.W; A.C.

Williams, Herbert Upham—

Si	Williams, Eliz. Sprague	Sociology	W.W.
FSiS	Sprague, Carleton	Finance; Art	W.W.

Welch, William Henry—

FSiS	Cowles, John Guiteau	Finance	W.W.
MBS	Collin, Frederick Welch	Law	W.W.
MBS	Collin, Charles Avery	Law	W.W.

Physicists

Abbe, Cleveland—

B	Abbe, Robert	Surg; Physics	A.M.S; W.W.
S	Abbe, Cleveland, Jr.	Meteorol; Geog.	A.M.S; W.W.
S	Abbe, Truman	Surg; Physiol.	W.W.
FSiS	Smith, Guilford	Finance; Philanth.	W.W.

Abbot, Charles Greeley—

FBS	Abbot, Henry Larcom	Eng; Warfare	A.M.S; W.W.
FBS	Abbot, Edwin Hale	Finance; Law	W.W.

Bauer, Louis Agricola—

B	Bauer, William C.	Elec. Eng.	A.M.S; W.W.

Bell, Alexander Graham—

F	Bell, Alex. Melville	Physiology	A.M.S; W.W; A.C.

Bell, Louis—

F	Bell, Louis	Warfare; Chem.	A.C.
FB	Bell, James	Politics; Law	A.C.
FB	Bell, John	Editing; Med.	A.C.
FB	Bell, Luther V.	Medicine	A.C.
FB	Bell, Samuel Dana	Law	A.C.
MB	Bouton, John Bell	Editing	A.C.
MF	Bouton, Nathaniel	Ministry	A.C.
FF	Bell, Samuel	Politics; Law	A.C.
FBS	Bell, Samuel Newell	Politics; Law	A.C.

Buckingham, Edgar—			
FF	Buckingham, Joseph T.	Editing; Publish.	A.C.
Crehore, Albert Cushing—			
B	CREHORE, WILLIAM W.	Bridge Eng.	W.W.
MSiS	CUSHING, HARVEY W.	Pathology	*A.M.S; W.W.
MSiS	CUSHING, HENRY PLATT	Geology	A.M.S; W.W.
MSiS	Cushing, William E.	Law	W.W.
Davis, Harvey Nathaniel—			
F	DAVIS, NATHANIEL F.	Mathematics	A.M.S; W.W.
Duane, William—			
B	Duane, Russel	Law	W.W.
FF	Duane, William	Publishing	A.C.
Franklin, William Suddards—			
B	FRANKLIN, EDWARD C.	Chemistry	*A.M.S; W.W.
Hering, Carl—			
B	HERING, RUDOLPH	Hydraulic Eng.	A.M.S.
Humphreys, William Jackson—			
FB	Humphreys, Milton W.	Philology	W.W; A.C.
Ives, Frederick Eugene—			
S	IVES, HERBERT EUGENE	Physics	A.M.S.
Jackson, Dugald Caleb—			
B	JACKSON, JOHN PRICE	Elec. Eng.	W.W.
B	Jackson, William B.	Engineering	W.W.
FSiS	Cravath, Paul Drennan	Law	W.W.
Kent, Norton Adams—			
B	Kent, William	Philanthropy	W.W.
Kimball, Arthur Lalanne—			
B	Kimball, A. Redington	Finance	W.W.
MB	Fisher, Samuel Ware	Ministry; Educ.	A.C.
MBS	Fisher, Samuel S., Jr.	Politics; Law	A.C.
Kinsley, Carl—			
F	Kinsley, William W.	Math; Theology	W.W.
Lyman, Theodore—			
F	LYMAN, THEODORE	Zoology	A.C.
FF	Lyman, Theodore	Philanthropy	A.C.
Magie, William Francis—			
F	Magie, William Jay	Politics; Law	W.W.
Mann, Charles Reborg—			
F	Mann, Charles H.	Inventing; Editing; Ministry	W.W.
FSi	Miller, Harriet Mann	Ornith; Writing	W.W.

Mendenhall, Thomas Corwin—

S	Mendenhall, C. E.	Physics	*A.M.S; W.W.

Mendenhall, Charles Elwood—

F	Mendenhall, T. C.	Physics	*A.M.S; W.W; A.C.

More, Lewis Trenchard—

B	More, Enoch Anson, Jr.	Fiction	W.W.
B	More, Paul Elmer	Poetry; Editing	W.W.
MF	Elmer, Lucius Q. C.	Politics; Law	A.C.

Northrup, Edwin Fitch—

B	Northrup, Elliott Judd	Law	W.W.
F	Northrup, Ansel Judd	Law	W.W; A.C.
FB	Northrup, William P.	Pathology	A.M.S; W.W.
MB	Fitch, Charles Elliott	Editing; Educ.	W.W.

Parson, William Barclay—

B	Parsons, Harry deB.	Mech. Eng.	A.M.S; W.W.

Rotch, Abbott Lawrence—

B	Rotch, Arthur	Architecture	A.C.
MF	Lawrence, Abbott	Diplomacy	A.C.
FBS	Rotch, Thomas M.	Pediatrics	*A.M.S; W.W.
MSiS	Lowell, Abbott Lawrence	Educ.	W.W.
MSiS	Lowell, Percival	Astronomy	*A.M.S; W.W; A.C.
MSiD	Lowell, Amy	Poetry	W.W.

Saunders, Frederick Albert—

B	Saunders, Arthur P.	Chemistry	*A.M.S; W.W.
B	Saunders, Charles E.	Chemistry	A.M.S.
B	Saunders, William E.	Botany	A.M.S.
F	Saunders, William	Horticulture	A.M.S.

Stevens, Walter LeCont—

MB	LeConte, John	Physics	A.C.
MB	LeConte, Joseph	Geology	W.W; A.C.
MF	LeConte, Lewis	Botany	A.C.
MBS	LeConte, Joseph N.	Mech. Eng.	A.M.S; W.W.

Stewart, Oscar Milton—

B	Stewart, George W.	Physics	*A.M.S; W.W.

Stewart, George Walter—

B	Stewart, Oscar M.	Physics	*A.M.S; W.W.

Trowbridge, Charles Christopher—

B	Trowbridge, S. B. P.	Architecture	W.W.
F	Trowbridge, W. P.	Engineering	A.C.

Very, Frank Washington—			
FB	Very, Jones	Lit; Ministry	A.C.
FSi	Very, Lydia Louisa A.	Literature	W.W; A.C.
Wead, Charles Dasson—			
MB	Kasson, John Adams	Politics	W.W; A.C.
Whitman, Frank Perkins—			
MSiS	Taylor, James Monroe	Educ; Ethics	W.W; A.C.
MSiD	Bissell, Mary Taylor	Medicine	W.W.
Wood, Robert Williams—			
MBS	DAVIS, BRADLEY MOORE	Botany	*A.M.S; W.W.
Wurts, Alexander Jay—			
B	Wurts, John	Law	W.W.
MF	JAY, JOHN CLARKSON	Medicine	A.C.
Zeleny, John—			
B	ZELENY, ANTHONY	Physics	*A.M.S; W.W.
B	ZELENY, CHARLES	Zoology	*A.M.S; W.W.
Zeleny, Anthony—			
B	ZELENY, CHARLES	Zoology	*A.M.S; W.W.
B	ZELENY, JOHN	Physics	*A.M.S; W.W.

Physiologists

Curtis, John Green—			
B	Curtis, Edward	Medicine	W.W; A.C.
B	Curtis, Joseph Bridgham	Warfare; Eng.	A.C.
½B	Curtis, George William	Lit; Publishing	W.W.
BD	Curtis, Constance	Art (Painting)	W.W.
BD	Curtis, Natalie	Music	W.W.
Dawson, Percy Millard—			
FB	Dawson, Samuel Edward	Hist; Publishing	A.C.
FSiS	ADAMS, FRANK DAWSON	Geology	A.M.S.
Hare, Hobart Amory—			
F	Hare, William Hobart	Ministry	A.C.
FF	Hare, George Enlen	Ministry	A.C.
MF	Howe, Mark A. DeWolfe	Ministry	W.W; A.C.
MBS	Howe, Mark A. DeWolfe	Editing	W.W.
Henderson, Yandell—			
MB	YANDELL, DAVID WENDEL	Surgery	A.C.
MF	YANDELL, LUNSFORD	Geology; Med.	A.C.
Hough, Theodore—			
FBS	HOUGH, WALTER	Anthropology	*A.M.S; W.W.

Lee, Frederic Schiller—			
B	LEE, LESLIE ALEXANDER	Zool; Geol.	*A.M.S; W.W.
B	Lee, John Clarence	Educ; Ministry	W.W.
F	Lee, John Stebbins	Educ; Ministry	W.W.
Lusk, Graham—			
F	LUSK, WILLIAM T.	Physiol; Med.	A.C.
MF	Chittenden, Simson B.	Finance; Politics	A.C.
Sewall, Henry—			
FF	SEWELL, THOMAS	Medicine	A.C.

Psychologists

Angell, James Rowland—			
B	Angell, Alexis Caswell	Law	W.W.
F	Angell, James Burrill	Educ; Diplomacy	W.W; A.C.
MF	CASWELL, ALEXIS	Astron; Educ.	A.C.
FBS	ANGELL, FRANK	Psychology	*A.M.S; W.W.
Angell, Frank—			
FB	Angell, James Burrill	Educ; Diplomacy	W.W; A.C.
FBS	Angell, Alexis Caswell	Law	W.W.
FBS	ANGELL, JAMES R.	Psychology	*A.M.S; W.W.
Bently, Madison—			
F	Bently, Charles E.	Ministry	W.W; A.C.
Bryan, William Lowe—			
B	Bryan, Enoch Albert	Education	W.W.
Cattell, James McKeen—			
B	CATTELL, HENRY WARE	Pathology	A.M.S; W.W.
F	Cattell, William	Educ; Ministry	A.C.
FB	Cattell, Alexander	Finance; Politics	A.C.
FBS	Cattell, Edward James	Econ; Geog; Lit.	W.W.
FBS	Cattell, William A.	Engineering	W.W.
Delabarre, Edmund Burke—			
B	Delabarre, Frank Alex.	Med; Dentistry	W.W.
Dewey, John—			
B	Dewey, Davis Rich	Econ; Statistics	W.W.
Hall, Granville Stanley—			
MBS	BEALS, EDWARD ALDEN	Meteorology	W.W.

Jastrow, Joseph—			
B	Jastrow, Morris	Theol; Sociol.	W.W.
F	Jastrow, Marcus	Philology	W.W.
Patrick, George Thomas White—			
Si	Patrick, Mary Mills	Educ; Writing	W.W.
Sanford, Edmund Clark—			
MSiS	SHINN, CHARLES H.	Botany	W.W.
MSiD	SHINN, MILICENT W.	Psychology	A.M.S; W.W.
Stratton, George Malcolm—			
B	Stratton, Frederick S.	Law	W.W.
Strong, Charles Augustus—			
F	Strong, Augustus H.	Educ; Theology	W.W; A.C.
Thorndike, Edward Lee—			
B	Thorndike, Ashley H.	Philology	W.W.
Wells, Frederic Lyman—			
F	Wells, Benjamin Willis	Language; Econ.	W.W.
Woodworth, Robert Sessions—			
½B	Woodworth, Frank G.	Educ; Ministry	W.W.
MB	Sessions, William R.	Politics; Agric.	W.W.

Zoologists

Andrews, Ethan Allen—			
B	ANDREWS, HORACE	Civil Eng.	A.M.S.
FF	Andrews, Ethan Allen	Lexicography	A.C.
Bruce, Charles Thomas—			
½B	Armstrong, William	Musical Criticism	W.W.
½Si	Benough, Elisa A.	Fiction	W.W.
Clark, Hubert Lyman—			
F	CLARK, WILLIAM S.	Chemistry	A.C.
MF	Richards, William	Education	A.C.
MBS	WILLISTON, ARTHUR L.	Mech. Eng.	W.W.
MBS	Williston, Samuel	Law	W.W.
Crampton, Henry Edward—			
MB	Miller, Charles Henry	Art (Painting)	W.W; A.C.
Dahlgren, Ulric—			
FB	Dahlgren, Ulric	Warfare	A.C.
FF	DAHLGREN, JOHN A.	Math; Warfare	A.C.
Dall, William Healey—			
F	Dall, Charles Henry A.	Ministry	A.C.
M	Dall, Caroline Wells H.	Lecturing; Lit.	W.W; A.C.

Davenport, Charles Benedict—			
B	Davenport, William E.	Sociol; Ministry	W.W.
Si	Davenport, Frances G.	History	W.W.
Drew, Gilman Arthur—			
B	Drew, William Lincoln	Law	W.W.
Forbes, Stephen Alfred—			
S	FORBES, ERNEST B.	Entomology	W.W.
BS	FORBES, ROBERT H.	Soil Chemistry	A.M.S; W.W.
Gage, Simeon Henry—			
Si	GAGE, MARY	Sanitation	W.W.
Gerould, John Hiram—			
B	Gerould, Gordon Hall	Philology	W.W.
B	Gerould, James	Library	W.W.
Glaser, Otto Charles—			
F	GLASER, CHARLES	Chemistry	A.M.S.
Grave, Caswell—			
B	GRAVE, BENJAMIN H.	Zoology	A.M.S.
Gulick, John Thomas—			
S	GULICK, ADDISON	Zoology	A.M.S.
F	Gulick, Peter Johnson	Ministry	A.C.
BS	GULICK, LUTHER H.	Physiol; Educ.	A.M.S; W.W.
BS	Gulick, Sidney Lewis	Ministry; Writing	W.W.
BD	Jewetts, Frances Gulick	Hygiene	W.W.
FBS	Gulick, Charles Burton	Philology	W.W.
Hargitt, Charles Wesley—			
S	HARGITT, GEORGE T.	Geology	A.M.S.
Herrick, Charles Judson—			
B	HERRICK, CLARENCE L.	Neurology	A.M.S.
Howard, Leland Ossian—			
MSiS	Stimson, Henry Lewis	Politics; Warfare	W.W.
MSiD	Keith, Dora Wheeler	Portrait Painting	W.W.
Jayne, Horace—			
B	Jayne, Henry La Barre	Law	W.W.
F	JAYNE, DAVID	Medicine; Pharmacy	A.C.
Lefevre, George—			
B	Lefevre, Albert	Philosophy	W.W.
B	Lefevre, Arthur	Education	W.W.

Lillie, Frank Rattray—			
B	LILLIE, RALPH STAYNER	Zoology	*A.M.S.
Lillie, Ralph Stayner—			
B	LILLIE, FRANK R.	Zoology	*A.M.S; W.W.
Loeb, Jacques—			
B	LOEB, LEO	Pathology	*A.M.S; W.W.
Mayer, Alfred Goldborough—			
F	MAYER, ALFRED M.	Physics	A.C.
FB	Mayer, Francis B.	Art	A.C.
Merriam, Clinton Hart—			
Si	BAILEY, FLORENCE M.	Ornithology	A.M.S; W.W.
FB	Merriam, Augustus C.	Archeol; Philol.	A.C.
Metcalf, Maynard Mayo—			
B	Metcalf, Irving Wight	Finance; Ministry	W.W.
B	METCALF, WILMOT V.	Chemistry	A.M.S.
FBS	Metcalf, Wilder S.	Warfare; Finance	W.W.
Montgomery, Thomas Harrison—			
B	Montgomery, James A.	Theol; History	W.W.
MB	Morton, James St. Clair	Warfare	A.C.
MB	MORTON, THOMAS G.	Surgery	A.C.
MF	MORTON, SAMUEL G.	Ethnol; Path; Anat.	A.C.
Moore, John Percy—			
B	MOORE, HENRY FRANK	Zoology	A.M.S; W.W.
Newman, Horatio Hackett—			
F	Newman, Albert Henry	History; Theol.	W.W.
Nutting, Charles Cleveland—			
MB	Hunt, Henry	Warfare	A.C.
MB	Hunt, Lewis Cass	Warfare	A.C.
Osborn, Henry Fairfield—			
B	Osborn, William Church	Law; Politics	W.W.
MF	Sturges, Jonathan	Trade	A.C.
Peckham, George Williams—			
FB	Peckham, R. Wheeler	Law	A.C.
FBS	Peckham, R. Wheeler, Jr.	Law	W.W; A.C.
FBS	Peckham, Wheeler H.	Law	W.W; A.C.
Rice, Edward Loranus—			
F	RICE, WILLIAM NORTH	Geology	*A.M.S; W.W; A.C.

Shufeldt, Robert Wilson—			
F	Shufeldt, Robert W.	Warfare	A.C.
Stone, Witmer—			
F	Stone, Frederick D.	History; Library	A.C.
True, Frederick William—			
B	TRUE, ALFRED CHARLES	Educ; Agriculture	A.M.S; W.W.
F	True, Charles Kitredge	Ministry	A.C.
Verrill, Addison Emery—			
S	VERRILL, ALPHEUS H.	Zoology	A.M.S; W.W.
Ward, Henry Baldwin—			
F	WARD, RICHARD H.	Bot; Microscopy	A.M.S; W.W; A.C.
FSi	Ward, Anna Lydia	Ethnol; Lexicog.	W.W; A.C.
Weed, Clarence Moores—			
B	WEED, HOWARD EVARTS	Entomology	A.M.S.
Zeleny, Charles—			
B	ZELENY, ANTHONY	Physics	*A.M.S; W.W.
B	ZELENY, JOHN	Physics	*A.M.S; W.W.

RELATIVES OF THE WIVES OF MEN OF SCIENCE

The same general plan used in the listing of the men of science and their distinguished relatives is followed below.

Anatomists

Donaldson, Mrs. Henry Herbert—			
F	Vaux, Calvert	Architecture	A.C.
MB	McEntee, Jervis	Architecture	A.C.

Anthropologists

McGee, Mrs. W. J.—			
F	NEWCOMB, SIMON	Astronomy	*A.M.S; W.W; A.C.
MB	Hassler, Ferdinand A.	Med; Literature	W.W.

Astronomers

Doolittle, Mrs. Charles Leander—			
B	Wolle, Fred	Music	W.W.
S	DOOLITTLE, ERIC	Astronomy	*A.M.S; W.W.
F	WOLLE, FRANCIS	Botany	A.C.

Frost, Mrs. Edwin Brant—			
F	Hazard, Marshal	Editing	W.W.
Holden, Mrs. Edward Singleton—			
B	CHAUVENET, REGIS	Mining Eng.	W.W; A.C.
B	CHAUVENET, WILLIAM M.	Chemistry	A.M.S; W.W.
F	CHAUVENET, WILLIAM	Mathematics	A.C.
Loud, Mrs. Frank Herbert—			
B	WILEY, WALTER H.	Mining Eng.	W.W.
Mitchell, Mrs. Samuel Alfred—			
F	DUMBLE, EDWIN T.	Geology	A.M.S; W.W.
Pickering, Mrs. Edward Charles—			
F	Sparks, Jared	History; Educ.	A.C.
MF	Silsbee, Nathaniel	Politics	A.C.
Pritchett, Mrs. Henry Smith—			
FB	McAllister, Julian	Warfare	A.C.
FF	McAllister, Nath. Hall	Law	A.C.
FBS	McAllister, Ward	Jurisprudence	W.W; A.C.
Wright, Mrs. William Hammond—			
F	Leib, Samuel	Law; Horticulture	W.W.

Botanists

Atkinson, Mrs. George Francis—			
F	KERR, W. C.	Geology	A.C.
Bessey, Mrs. Charles E.—			
S	BESSEY, ERNST A.	Botany	*A.M.S; W.W.
Clements, Mrs. Frederic Edward—			
Si	Schwartz, Julia	Literature	W.W.
Coulter, Mrs. John Merle—			
S	COULTER, JOHN G.	Botany	A.M.S.
Coulter, Mrs. Stanley—			
B	Post, Roswell Olcott	Ministry	W.W.
FB	Post, Truman	Ministry	A.C.
Farlow, Mrs. William Gilson—			
Si	Horsford, Cornelia	Archeology	W.W.
F	HORSFORD, EBEN N.	Chemistry	A.C.
M	Horsford, Mary L. H.	Poetry	A.C.
FF	Horsford, Jedediah	Warfare; Politics	A.C.
Ganong, Mrs. William Francis—			
B	Carman, Bliss	Poetry; Editing	W.W.

Greenman, Mrs. Jesse More—			
MSiS	Hartranft, John F.	Politics	A.C.
Pinchot, Mrs. Gifford—			
F	Bryce, Lloyd S.	Editing; Politics	W.W.
MF	Cooper, Edward	Finance; Politics	W.W.
Ramaley, Mrs. Francis—			
F	JACKSON, EDWARD	Ophthalmology	W.W.
Rose, Mrs. Joseph Nelson—			
FB	Sims, Charles R.	Ministry; Educ.	A.C.
Stone, Mrs. George Edward—			
F	CLARK, HENRY JAMES	Botany	A.C.
Wilson, Mrs. William Posell—			
FF	Williams, Charles K.	Politics	A.C.

Chemists

Bigelow, Mrs. Samuel Lawrence—			
MF	Harrison, Joseph	Railroad Building	A.C.
Burgess, Mrs. Charles Frederick—			
B	Jackson, Charles F.	Literature	W.W.
Cushman, Mrs. Allerton Seward—			
B	Hoppin, Joseph Clark	Archeology; Art	W.W.
FB	Hoppin, Augustus	Art; Literature	A.C.
FB	Hoppin, Thomas	Art; Sculptoring	A.C.
FB	Hoppin, William Jones	Stage; Editing	A.C.
Franklin, Mrs. Edward Curtis—			
B	Scott, Charles Fred.	Politics	W.W.
Gies, Mrs. William John—			
MB	Tressler, David	Education	A.C.
MBS	Tressler, Victor	Education	W.W.
Kahlenberg, Mrs. Louis—			
B	HEALD, FRED. DEFOREST	Botany	*A.M.S; W.W.
Long, Mrs. John Harper—			
FB	Stoneman, George	Warfare	A.C.
Marshall, Mrs. John—			
F	WORMLEY, T. G.	Chemistry	A.C.

Munroe, Mrs. Charles Edward—			
F	BARKER, GEORGE FRED.	Physics	*A.M.S; W.W; A.C.
Osborne, Mrs. Thomas Burr—			
F	JOHNSON, SAMUEL WM.	Chemistry	*A.M.S; W.W; A.C.
Palmer, Mrs. Chase—			
FBS	Edwards, Howard	Educ; Linguistics	W.W.
FBD	Edwards, Louise	Literature	W.W.
Pellew, Mrs. Charles Ernest—			
F	CHANDLER, CHARLES F.	Chemistry	*A.M.S; W.W; A.C.
FB	CHANDLER, WILLIAM H.	Chemistry	A.M.S; W.W; A.C.
Richards, Mrs. Theodore Wm.—			
F	Thayer, Joseph Henry	Theology	W.W; A.C.
Sanger, Mrs. Charles Robert—			
F	Davis, Andrew	Numismatics	W.W; A.C.
FB	Davis, Hasbrook	Warfare	A.C.
FB	Davis, Horace	Manufacturing	A.C.
FB	Davis, John C. B.	Diplomacy	A.C.
FF	Davis, John	Politics	A.C.
FBS	Davis, John	Law; Diplomacy	A.C.
Saunders, Mrs. Arthur Percy—			
F	Brownell, Silas B.	Law	W.W.
Shimer, Mrs. Porter William—			
B	Sandt, George W.	Ministry; Editing	W.W.
Talbot, Mrs. Henry Paul—			
FSiS	Baker, Newton D.	Law; Diplomacy	W.W.
Tassin, Mrs. Wirt—			
F	Moran, Thomas	Art; Exploration	W.W; A.C.
M	Moran, Mary Nimo	Art	A.C.
FB	Moran, Edward	Art	A.C.
FB	Moran, Peter	Art	W.W; A.C.
FBS	Moran, John Leon	Art	W.W; A.C.
FBS	Moran, Edward Percy	Art	W.W; A.C.
FBD	Moran, Annette	Art	W.W.
Van Slyke, Mrs. Lucius L.—			
S	VAN SLYKE, DONALD D.	Chemistry	A.M.S.

Venable, Mrs. Francis Preston—			
B	Manning, James S.	Jurisprudence	W.W.
FB	Manning, Thomas C.	Jurisprudence	A.C.
Wiechman, Mrs. Ferdinand G.—			
B	Damrosch, Frank	Music	W.W; A.C.
B	Damrosch, Walter	Music	W.W; A.C.
F	Damrosch, Leopold	Music	A.C.
Wiley, Mrs. Harvey Washington—			
F	Kelton, John C.	Warfare; Sociol.	A.C.

Geologists

Brooks, Mrs. Alfred Hulse—			
F	BAKER, FRANK	Anatomy	*A.M.S; W.W.
Chamberlin, Mrs. T. C.			
S	CHAMBERLIN, R. T.	Geology	A.M.S; W.W.
Cross, Mrs. Whitman—			
FBS	Stevens, Isaac I.	Exploration; Eng.	A.C.
Eastman, Mrs. Charles—			
F	CLARK, ALVAN G.	Astronomy	A.C.
FF	CLARK, ALVAN	Astronomy	A.C.
Gannet, Mrs. Henry—			
FSiS	Gordon, Seth C.	Surgery	W.W.
MSiS	Howe, Lucien	Ophthalmology	W.W.
Grant, Mrs. Ulysses Sherman—			
B	WINCHELL, ALEX. N.	Geology	*A.M.S; W.W.
B	WINCHELL, HORACE V.	Geology	A.M.S; W.W.
F	WINCHELL, NEWTON H.	Geology	*A.M.S; W.W; A.C.
FB	WINCHELL, ALEXANDER	Geology	A.C.
FB	Winchell, Samuel R.	Educ; Lit.	W.W.
Harris, Mrs. Gilbert Dennison—			
FB	Stoneman, George	Warfare	A.C.
Hitchcock, Mrs. Charles Henry—			
F	Barrows, E. P.	Linguistics	A.C.
Hobbs, Mrs. William Herbert—			
BS	Kimball, Alonzo	Art	W.W.
FSiS	BANISTER, HENRY	Geol; Med.	A.M.S.
Mathews, Mrs. Edward Bennett—			
B	WHITMAN, FRANK P.	Physics	*A.M.S; W.W.

Pirsson, Mrs. Louis Valentine—			
F	BRUSH, GEORGE JARVIS	Mineralogy	*A.M.S; W.W; A.C.
Pumpelly, Mrs. Raphael—			
D	Smyth, Margarita P.	Art	W.W.
SiS	Hill, Edward B.	Music	W.W.
MBS	Pope, Alexander	Painting	W.W.
Rice, Mrs. William North—			
S	RICE, EDWARD L.	Zoology	*A.M.S; W.W.
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FB	Garland, Hugh A.	Law; Warfare	A.C.
FBS	Garland, Hugh A.	Law; Warfare	A.C.
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BS	Ewing, Nathaniel	Jurisprudence	W.W.
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FF	Upham, Charles W.	Lit; Theol; Polit.	A.C.
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F	Hill, Ebenezer J.	Politics	W.W.
Willis, Mrs. Bailey—			
F	BAKER, FRANK	Anatomy	*A.M.S; W.W.
Winchell, Mrs. N. H.—			
S	WINCHELL, ALEXANDER	Geology	*A.M.S; W.W.
S	WINCHELL, HORACE V.	Geology	A.M.S; W.W.

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B	Burton, Ernest DeWitt	Theology	W.W.
B	Burton, Henry	Linguistics; Educ.	W.W.
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MB	Tucker, Nathaniel B.	Law; Literature	A.C.
MF	Tucker, St. George	Linguistics	A.C.
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MB	HOPKINS, FRED V.	Medicine	A.C.
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FBS	Shaw, Robert G.	Warfare	A.C.
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MSiS	CUSHING, HENRY PLATT	Geology	A.M.S; W.W.
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FF	Hodge, Charles	Theology	A.C.
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SiD	Taft, Helen Herron	Education	W.W.
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MB	JONES, FREDERICK S.	Physics	*A.M.S; W.W.

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F	MELTZER, SAMUEL J.	Physiology	*A.M.S; W.W.
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F	Taylor, Hannis	Law; Lit; Diplom.	W.W; A.C.

Lusk, Mrs. Graham—			
F	Tiffany, Louis C.	Inventing; Art	W.W; A.C.
FF	Tiffany, Charles L.	Finance; Mfg.	W.W; A.C.

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F	Bayard, Thomas F.	Diplomacy	A.C.
FF	Bayard, James A.	Diplomacy	A.C.
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FB	Rockefeller, William	Finance	W.W; A.C.
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S	GULICK, JOHN ADDISON	Zoology	A.M.S.
Hargitt, Mrs. Charles Wesley—			
S	HARGITT, GEORGE T.	Zoology	A.M.S.
Jayne, Mrs. Horace—			
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B	FURNESS, WILLIAM H.	Anthropology	A.M.S; W.W.
F	Furness, Horace H.	Literature	W.W; A.C.
M	Furness, H. K.	Literature	A.C.
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Linton, Mrs. Edward—			
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Si	Brownson, M. Wilson	Math; History	W.W.
MB	Acheson, Marcus W.	Jurisprudence	W.W.
MBS	Acheson, Alex. W.	Med; Politics	W.W.
MBS	ACHESON, EDWARD G.	Chemistry	*A.M.S; W.W.
MBS	Acheson, Ernest F.	Editing; Politics	W.W.
MBS	Acheson, Marcus W.	Jurisprudence	W.W.
Mast, Mrs. Samuel Ottmar—			
B	TENNENT, DAVID HILT	Biology	*A.M.S; W.W.
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Newman, Mrs. Horatio Hackett—			
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MSiS	LILLIE, RALPH	Physiology	*A.M.S.
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Sedgwick, Mrs. William Thompson—			
B	Rice, Richard Austin	Languages; Hist.	W.W.
Shufeldt, Mrs. Robert Wilson—			
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Stiles, Mrs. Charles Wardell—			
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MF	Curtis, William S.	Education	A.C.
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Verrill, Mrs. Addison Emory—			
B	Smith, Sidney	Zoology	*A.M.S; W.W.
S	Verrill, Alpheus H.	Zoology	A.M.S; W.W.
Wilson, Mrs. Henry Van Peters—			
MB	Stedman, Henry Rust	Pathology	A.M.S; W.W.
Zeleny, Mrs. Charles—			
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Lewis, Margaret Reed	Biology	A.M.S.

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McGee, Anita Newcomb	Medicine	A.M.S; W.W.

Botanists

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Wilson, Lucy M. Williams	Biology	A.M.S; W.W.

Chemists

Norton, Thomas Herbert—		
Norton, Edith Eliza Ames	Literature	W.W.
Phelps, Isaac King—		
Phelps, Martha Austin	Chemistry	A.M.S.

Geologists

Grabau, Amadeus William—		
Grabau, Mary Antin	Literature	W.W.

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Franklin, Christine Ladd	Psychology	*A.M.S; W.W.
Peirce, Charles Santiago Sanders—		
Peirce, Harriet M. Fay	Literature	W.W.

Pathologists

Cabot, Richard C.—		
Cabot, Ella Lowell Lyman	Philos; Education	W.W.

Physicists

Barus, Carl—		
Barus, Annie G. Howes	Literature	W.W.

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ANDREWS, SARA G. FOULKE	Zoology	A.M.S.
Calvert, Philip Powell—		
CALVERT, AMELIA K. SMITH	Zoology	A.M.S.
Comstock, John Henry—		
COMSTOCK, ANNA BOTSFORD	Entomology; Art	A.M.S; W.W.
Davenport, Charles Benedict—		
DAVENPORT, GERTRUDE CROTTY	Zoology	A.M.S.
Gage, Simon Henry—		
GAGE, SUSANNA PHELPS	Zoology	*A.M.S; W.W.
Jayne, Horace—		
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PECKHAM, ELIZ. M. GIFFORD	Zoology	A.M.S; W.W.
Wilder, Harris Hawthorne—		
WILDER, INEZ WHIPPLE	Zoology	A.M.S; W.W.

(*To be continued*)

AUTOPHORIC TRANSPLANTATION, ITS THEORY AND PRACTISE

PROFESSOR HANS PRZIBRAM

Biologische Versuchsanstalt der Akademie der Wissenschaften, Vienna

If a machine breaks down, the mechanical engineer has four ways of repairing it. He may discard the broken parts and reconstruct the whole on a smaller scale; he may fabricate the missing part and fit it into its right place again; he could also take a piece from another machine of more or less similar type, as long as an exchange is made possible by the material of the parts, soldering the broken pieces together or fixing them by screws, wires, etc.; or lastly he may simply exchange the broken part for a whole one, first taking the former out of the broken machine at the points where it was joined, and refitting the new part, taken in like manner out of a similar machine by the same means, in the place of the first. An organism is often just as badly in want of repair as a machine of human fabric. In comparing the two I do not wish to enter here into the controversy of Mechanism *versus* Vitalism. No vitalist will deny that the body of an animal, let us say of vertebrate or arthropod type, is built up of various contrivances the physicist calls machines, and that its functions are best described in physical and chemical terms. It is not the machinery of organized forms that he would throw doubt on, but the mechanical or chemical nature of its driver. Now, when living machinery is broken or maimed, there are the same four possibilities of repair stated above. The organism may shed such parts as are now superfluous for its reduced size and reconstruct itself on the basis of a proportionately diminished form, as in small pieces of planarians, a process called "Morphallaxis," by T. H. Morgan. Secondly, a missing part may be

manufactured anew by the remaining body of the animal, such "Regeneration" not being uncommon even in whole extremities of amphibians and crayfish. But, unfortunately, in the warm-blooded vertebrates this faculty is very limited, not extending much beyond the repair of small pieces of tissue, and never including a whole organ or appendage. It has therefore long been customary in human medicine to try to replace lost parts by "transplantation" of cornea, skin, muscle, bone, or even nerve and blood vessel. Without regard to the composition of the injured part, small pieces or larger portions have been taken from the same or from another individual, and again without special orientation have been grafted upon the wound. All sorts of fastenings have been tried, bandages, plaster, wires, ligatures, but mostly with poor results. The same methods and many others have been applied in experimental zoology, but only when embryonic stages which had not functioned before the operation were used have good results been achieved. Nevertheless it has been demonstrated by A. Carrel that even whole limbs and kidneys may be again healed back in mammals and in the case of the latter again become functionally active. But the tedious method of sewing every sinew, blood vessel and nerve together seems to have prevented till now the general application of this discovery. Carrel's method, as also that of other surgeons, must be compared to the third method of the engineer, when he is soldering or fixing a broken piece on to another, trying to repair the machine without taking it to pieces. Now it is generally simpler to take out the injured piece of a machine, by unscrewing or unsoldering or even by striking it out of the whole by sheer force, so that its connections give way at the points of least resistance, and to replace it by a new one of exactly the same form, than to try and fix the broken parts together again at the point of breakage. Is there a possibility of applying this fourth method of the engineer to the organism? One will, perhaps, at first be inclined to doubt this proposi-

tion. The vitalist will now come forward and claim that the organism is not constituted by parts simply fastened together at certain points, that its unity is the cause of its function; the mechanist will be inclined to doubt the possibility of whole organs regaining their function by "exchange" in animals without high regenerating power, for he has been trained to believe in the destruction of function by the severing of the nerve.

Let us turn to facts. Certain animals, widely distributed through the animal kingdom, practise the faculty of shedding appendages or other parts of their body at certain preformed breaking points. This "autotomy" is also observed in the Crinoid, *Antedon rosaceus.* Working at the Naples Station in 1900 on the regeneration of these Crinoids I wanted to find out if the color in regenerating arms would be influenced by the color of the visceral mass. Now *Antedon* shows a great variety of very distinct shades, such as bright yellow, carmine red and chocolate brown. The visceral mass, easily shed by the animal, was transplanted in proper orientation to a specimen of different color, also void of its viscera. It was immediately accepted by the new owner and clutched tightly to the calyx, as is the usual thing with the normal animal. The connections between the new visceral sac and the body were soon restored, the exchange succeeding in every case. Mouth and anus, both situated on the surface of the visceral sac, became functional again. It is clear that here there is a case of the fourth method of the engineer, namely the replacement of a missing part by a new one of exactly the same form fixed in at the same connecting points as before. One difference is apparent: in the machine there will be little if any activity on the part of the receiver or the new part, whilst in the Crinoid the newly fixed parts are reunited by internal forces. If we want to understand the "exchange" followed by function, it is therefore necessary to know the nature of these forces. Is it possible to account for them on the ground of our present knowledge of living matter? Can we con-

ceive the organism as an engineer mending his own body? When the visceral mass of *Antedon* is not replaced, a new sac is regenerated by the creature. As in all cases of regeneration known to me, it is nothing else than an acceleration of growth going on normally at slower rate, but in the same direction and sense. From this theoretical standpoint, which has been proved to be correct over and over again, we can be satisfied that there are growing forces in the *Antedon* sufficient to ensure the attachment of the new visceral sac.

We have heard that in higher animals regeneration is not as ready to supply lost parts, and as soon as growth ceases, for instance in the imago of insects, the faculty of restoring missing limbs is lost. But a certain degree of repair has been noticed and experimentally tested even here, for instance the closing of holes pricked in the integument of beetles, and even the resprouting of torn-out wings as mere skin duplicatures. In vertebrates a good deal of physiological regeneration is always going on in the tissues, and transplanted pieces of living tissue often become attached in a short time by connective tissue and blood vessels growing over and into them. Will exchange of organs lead under certain conditions to their functional restoration also in such animals as these? The first condition must be the possibility of removing the part to be replaced always in the same place and manner, so as to be sure that it will comprise just the same material and fit in again in the corresponding place of the new host. Planes of preformed breakage would answer best to this condition, but they are generally precluded by the second condition that must be fulfilled, namely retention of the implanted organ by the own forces of the recipient. Such forces may be divided into three groups: first, the natural friction of a mass pressed into a socket, also aided by atmospheric pressure; secondly, the active aid of muscle and nerve clutching the implanted organ and preventing it from falling out of its place; thirdly, the clotting of the body fluids, gluing, as

it were, the graft to the stock. During the last two years my pupils and myself have tried to extend this method, which I now call "autophoric" or self-retaining transplantation, to other cases than the visceral sac of *Antedon,* and we have found that under these conditions function can be restored in a degree unknown till now, at least in developed animals.

The eye of vertebrates may be described as a ball-shaped camera movable by three pairs of levers in all directions of space, connected with its supply of chemicals by the blood vessels and in communication with its operator, the brain, by the optic nerve. If these fixing strings are severed, there is scarcely any attachment to the surroundings save some connecting tissue of unspecialized sort. The "camera" itself will not be injured, if the whole eyeball be taken out of the orbit, and there is scarcely a possibility of altering the points of severance if the enucleation be made quickly and with decision. If the eye is restored to its orbit, it will therefore be possible for all the above-mentioned connections to join again. This was observed as long ago as 1906 by Ruggero Pardo in *Triton,* who made experiments on the necessity of the presence of the optic nerve for the regenerative process in the eye of this amphibian. Unintentionally he had excised the eyeball with the nerve and was much astonished at its reattachment to the orbit. But will eyesight be restored with this reattachment? Pardo was not able to convince himself of this fact, although on histological examination he found the optic nerve regenerated. I have suspected for some time that the vertebrate eye might furnish good material for the restoration of function by autophoric transplantation, as it will in many forms be retained in the orbit by friction and atmospheric pressure alone, aided also in some cases by the eyelids closing over the eyeball, and by its great surface securing wide contact with the blood issuing into the orbit after extirpation. My own first experiments to realize this expectation in new-born rats failed.

In the new-born rat, as in many mammals, the eyes are tightly closed and the lids connected by tissue. This seemed to afford favorable conditions for the exchange of eyes, as they would be kept in place by the tight closure of the eyelids. Having severed the lids, I interchanged the eyes and, as expected, the eyelids shut again tightly and kept the eyeballs in place. But when the eyelids opened again at the normal time, the eyes had grown on, although they were not functional, and totally disappeared in time. Disappointed at this failure, the experiments were discontinued. It is now pretty certain that this poor result was due to the unfavorable conditions obtaining in very young mammals, for we are now able to demonstrate the correctness of my original supposition. Theodor Koppányi, a young Hungarian student, working under my direction in the "Biologische Versuchsanstalt" in Vienna, has succeeded in making the autophoric transplantation of the eye in a variety of species, extending from fish to mammal. The work of Pardo on *Triton* was confirmed, and older rats yielded excellent results. It seems that in the young stages of rats there were difficulties in the way of the eye obtaining a sufficient supply of blood, since also in Koppányi's experiments it was far easier to get the eyes to become reattached and functional in older specimens.

Indeed, it is probable that the pressure of the eyelids exerted on the replaced eyeball in the new-born rats is a hindrance. Grown rats do not close the eyelids tightly upon the eyeballs, so that it is even advisable to pin the lids or sew them together for a day or two, lest the animal whisk out the implanted eyes or scratch at them before they are attached sufficiently firmly to withstand such treatment.

We have been able to show that these replanted eyes are functional, all possible tests yielding positive results and being in striking contrast to those in blinded animals.[1] Microscopical examination of sections through

[1] For details of these experiments I must refer to our previous short

replanted eyes, which had again regained their function, has been made by Professor Walter Kolmer, of the Physiological Institute, University of Vienna, and the re-ingrowth of the severed optic nerve-fibers into the optic thalamus is beyond doubt. Professor Kolmer, as all other authorities, to whom the animals with functioning replanted eyes were shown, stated that they would scarcely have believed the fact, without having themselves seen and tested it. Some oculists even refused to believe what they saw, taking refuge in far-fetched explanations for the absolutely normal behavior of the rats and for the connection of retina and brain in anatomical and microscopical preparations. But is the restoration of function in the vertebrate eye really in contradiction to the facts known to us concerning the regeneration in this animal type? If we resort to our theory of regeneration as accelerated growth, moving on the same lines as normal differentiation, and waning with higher specialization, it is necessary to inquire into the normal development of the eye and optic nerve, before answering this question. The vertebrate eye grows from multiple origins, the nervous elements being derived from a fold of the central nervous system (brain). It is generally believed that the nerves of the brain grow in centrifugal direction and are incapable of regeneration, as one does not observe regeneration-cones at the peripheral end of sectioned central nerves as a rule. Ramón y Cajal, on the other hand, thinks that this inability to regenerate is only a consequence of secondary difficulties, regeneration at least commencing when the right nurture is given: this may be accomplished by inserting degenerating nerve-pieces into the pathway of the sectioned nerve. At any rate there would be but little chance of quick and sufficient regeneration, if the eye depended on the nerve growing into it from the brain. Fortunately, as is well known, the fibers of the optic nerve in ontogeny grow

communications in the *Akademischer Anzeiger*, Wien; they will be followed by publication in extenso in the *Archiv für Entwicklungsmechanik*, 1922.

centripetally from the retina towards the thalamus opticus. In regeneration this same process need only be repeated. Edward Uhlenhuth, while working at our "Biologische Versuchsanstalt," proved in 1912 that the optic nerve of salamander eyes implanted on the back of the same species grows centripetally towards the spinal cord and even in several instances united with the next spinal ganglion. These transplanted eyes were of course devoid of function, as the nerve had not reached its proper center, but it was of greatest interest to note that the eye, although severed and removed from its natural connection, had totally regenerated after a short period of partial degeneration. Bearing these two points in view, the centripetal growth in ontogeny and the same process in transplanted eyes, we see our theoretical demands for the reattachment of replanted eyes fulfilled: the nerve fibers will grow backwards through the orbit, continuing on their usual path and probably finding good conditions there in the degenerating central stump. The usual assumption that function of a sensitive organ can not be restored after severing the nerve is based on false presumptions, especially the idea that the proper central nerve center is responsible for regeneration. We have in several instances proved that it is not necessary for a body part to be connected with its normal nervous center for regeneration to set in and proceed till completion. I may call attention to Oskar Kurz's transplantations of knees taken from developed tritons and placed on the side of the same animal. Out of this bit of leg all distal parts were regenerated, tibia, fibula, foot and toes, although connection of the nerve-stump remaining in the graft with the normal nervous center in the lumbar region can not have taken place. It is quite another question, how far the presence of nerve is necessary for restoration of normal form; a question often confounded with the inability of reestablishing function after severing of nerves. I will not enter into these problems here, as they are being investigated by several of my fellow-workers

and definite statements can not yet be made. The foundation for the statement that eyes severed from their connection with the brain are not able to regain sight seems to lie in the fact that the optic nerve in mammals, when the eyes are left movable by their proper muscles, can not find its way to a connection with any nerve center, and then degenerates with the other parts of the eye. It seems that the regenerating ends of the optic nerve fibers coming from the retina are carried to and fro by each rolling of the eye and thus fail to connect with the central stump of the nerve. In contrast to this sheering of the fibers in eyes left attached to the orbit after severing of the nerve, the nerve fibers in autophoric replantation reach their goal before the muscles have grown together and become movable again. It must be emphasized that our method involves no injury to the nerve besides a clean cut, and also that Boeke in Amsterdam has been able to obtain results in nerve regeneration far exceeding those of previous experimenters by avoiding suturing or otherwise ill-treating the nerves.

A second opportunity for autophoric replantation is afforded in the vertebrate eye by the lens. It is well known that this part of the eye is derived ontogenetically from an invagination pinching off from the outer layer of ectoderm. The lens of cold-blooded vertebrates, especially urodeles, is capable of regeneration and is easily extracted as a whole, and when it is replanted again into its former place, it fits well into the lens-sac. At my suggestion Berthold Wiesner has applied the method of autophoric replantation to the lens of fish and amphibia; the results show that replanted lenses can clear up again and restore normal eyesight to their bearer. In mammals analogous experiments have not yet succeeded, perhaps because in the rat, the only available mammals for the present, conditions are unfavorable in respect to the relative size of lens, cornea and eyeball. In other forms, as in man, where the lens relative to the size of the eye is much smaller, replantation should succeed, as

the retraction often practised by the oculist is easy, and even regeneration of the lens has been occasionally recorded (see Literature, Przibram, Regeneration, 1909).

Unlike the eye of vertebrates, arthropod eyes are not suitable for our method of transplantation. They usually protrude much too far from their socket to be kept in place after their replantation solely by the friction or other forces exerted by the host. A discovery of Walter Finkler has nevertheless put us in position to avail ourselves of the autophoric method for furnishing insects with a new pair of eyes. This young student, having had the opportunity of seeing the results in vertebrates, severed the head of several types of hexapodes from the thorax and, replanting it on its own body or on that of another decapitated individual, observed its retention by the friction and blood clot. There can be no doubt that also in these cases function is restored, all reactions of the normal animal reappearing after a few days or weeks, and the tissues joining quickly. Finkler has worked on the larval, pupal and imaginal state. Perhaps the most astonishing fact is the ready response of the imago to such operations in spite of its lack of regenerative power. But also in this case, as in the higher vertebrates, we shall have to take into account that in our experiments no other processes of reparation are called into play than those of slow physiological regeneration, which still persist in adult organisms. At any rate, in all the tissues of adult insects severed connections are quickly restored, when the organs are left in place, as Finkler could prove. His experiments on autophoric transplantation in insects will be extended to appendages, whilst P. Weiss, Koppányi, Finkler and Wiesner are also occupied with autophoric replantation in parts of the vertebrate body other than the eyes.

Summary

1. Well-defined parts of the animal body that may be easily detached at the same connecting points can be replaced by similar new organs under following conditions:

(*a*) equal size and orientation; (*b*) simple exchange without exertion of pressure or additional injury to the nerve beyond a clean cut; (*c*) prevention of loss by the natural means of the animal itself (friction, clasp, blood clot).

2. By this method of "autophoric" or "self-retaining" transplantation, the graft taken from an adult individual and replanted into another may be restored to function, even the nerves of the head reuniting, and the bearer being repaired in every respect.

3. These achievements are in accord with the theory stating regeneration to be nothing else than the acceleration of physiological processes going on all the time in the body of organisms, for it can be demonstrated that the reattachment proceeds in the same sense as the first growth of the nerve. They contradict, however, the general assumption that the maintenance and functional regeneration of organs are dependent on their uninterrupted connection with their special nervous center.

4. Till now we have been able to obtain autophoric replantation with restoration of function in the visceral sac of *Antedon* (Echinoderms—*Przibram, 1901*), in the eyes of fish, amphibia and mammals (Vertebrates—*Koppányi, 1921*), in the lens of the two former classes (*Wiesner, 1921*), in the heads of insects, walking sticks, water bugs, water beetles (Insects—*Finkler, 1921*) and in other cases not yet ready for publication.

5. Experiments with larval stages of amphibia and insects as compared with the imaginal state of the same species show that there is no radical difference as to the restoration of function after excision and replantation of a part, in mammals (rats) grown-up specimens even seeming to be more favorable for autophoric replantation.

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SPONTANEOUS METAMORPHOSIS OF THE AMERICAN AXOLOTL

PROFESSOR W. W. SWINGLE

OSBORN ZOOLOGICAL LABORATORY, YALE UNIVERSITY

THE following experiments on axolotl neoteny and metamorphosis are published, not because of the conclusive nature of the results obtained, but the reverse—because of their inconclusiveness. A record of the work seems warranted in order that other investigators of this problem may be spared considerable expense, time and effort due to unsuitability of the material for experimentation.

The latter part of April, 1922, one hundred and nine axolotl larvæ of *Amblystoma tigrinum* were received from Albuquerque, New Mexico. These animals were obtained through the courtesy of Mr. J. N. Gladding. They varied in length from four inches to fourteen inches, though the average total length was about seven inches. One animal measured fourteen inches from snout to tail tip, another measured eleven inches. They were the largest individuals of the lot. The animals were in excellent condition on arrival and none showed any indications of metamorphosis.

EXPERIMENT 1. AUTOPLASTIC THYROID TRANSPLANTATION

May 5, 1922, the thyroids of seven axolotls, seven inches in length, were removed under chloretone anesthesia and each gland transplanted intraperitoneally into the same individual from which it was taken. The idea was that the acquisition of a new blood and nerve supply by the gland in its new environment might permit the release of the accumulated secretion and so metamorphose the animal. It was shown by the writer ('21) that the thyroid glands of axolotls are highly active metamorphosis-inducing agents providing the hormone escapes into the

blood stream. In these forms there appears to be some inhibition of the secretory (excretory) functions of the thyroid, and the hormone is retained within the gland vesicles.

The experimental animals and their controls were kept in large aquaria with plenty of water and food. One of the grafted animals had metamorphosed by June 27. Three others transformed by July 1; a fifth animal died without transforming July 6. The two remaining axolotls had not metamorphosed by September 1. During the interval between May 21 and September 1, all of the controls spontaneously transformed. The experiment is, of course, without significance because of the unstable nature of the control material. It is highly probable that the operated animals would have metamorphosed just about as rapidly if the thyroid had been left in its normal position.

Experiment 2. Homoplastic Thyroid Transplantation

Five seven-inch axolotls were engrafted intraperitoneally with the thyroid gland of other animals of similar size and appropriately controlled by animals transplanted with pieces of muscle tissue.

The transplants were made May 2, 1922. One animal had transformed by June 3, a second by June 6, a third June 11. Two animals remained as larvæ and were reengrafted June 11 with axolotl thyroids, and metamorphosed by July 3. In the meantime the controls also transformed. A large series of transplantation experiments were performed, using various endocrine glands, but in every case except two experiments the controls metamorphosed along with the operated individuals.

Heteroplastic Thyroid Transplantation

Four eight-inch axolotls were engrafted intraperitoneally with the glandular tissue of adult *Necturus maculatus*. Each axolotl received the entire thyroid of a single

Necturus. The experiment was performed May 15. By June 11 all of the engrafted animals had transformed but none of the controls for this particular group, though normal, untreated animals used as checks for other experiments were metamorphosing during this interval.

Despite the unstable nature of the control material used, this experiment seems fairly sound and indicates that *Necturus* thyroids when injected in sufficient quantity will metamorphose axolotl. To be absolutely reliable this experiment should have been performed upon thyroidectomized forms, but unfortunately the unsuitable nature of the controls was not known until too late.

Thyroid Feeding Experiments

Five six-inch axolotls were fed desiccated thyroid tissue (Parke, Davis and Company), containing 0.21 per cent. iodine by weight. The feeding was done by means of a pipette May 18. Two animals had transformed by May 27, and all by June 10. None of the controls metamorphosed during this interval but all transformed by July 25. The experiment seems trustworthy, especially in view of similar results obtained by other investigators on animals of the European strain.

Heteroplastic Pituitary Transplantation

Five axolotls varying in length from four to seven inches were each grafted with two whole pituitary glands of adult *Rana clamata* frogs. The grafts were made May 5. June 3 one animal metamorphosed; June 7 a second transformed. June 10 the three remaining animals were reengrafted with frog pituitaries. All metamorphosed by June 25.

During the interval between May 5 and June 25 only two of the controls for this particular group transformed, but it must be remembered that control animals of other cultures were metamorphosing. The experiment is recorded for what it is worth, but the writer believes that

injection of fresh pituitary substance does induce axolotl metamorphosis possibly by serving to release the thyroid hormone. This experiment should be tried on the Mexican strain of axolotl which apparently rarely spontaneously metamorphoses and hence can be safely controlled.

Thyroidectomy and Metamorphosis

Eight axolotls varying from seven to fourteen inches were thyroidectomized and at the present writing, September 1, are still larvæ and show no indications of transforming. Out of the original one hundred and nine animals received from New Mexico these eight are the only ones that have not metamorphosed. It is a fairly safe assumption that these axolotls will remain permanently as larva now that the thyroid gland is lacking.[1]

The thyroids of several animals were removed after the onset of metamorphosis, *i.e.*, after the tail fin and gills were undergoing reduction, but in all cases the removal of the thyroid failed to prevent the completion of metamorphosis.

Discussion

The conclusion to be drawn from these experiments is that the New Mexican strain of axolotl is entirely too unstable to work with on any problem involving the methods of feeding, injection or transplantation, where the results require a lapse of several weeks to obtain. The animals can not be controlled when the thyroid apparatus is left intact. It is evident that conclusive experiments of the above kinds on the New Mexican strain of axolotl (where the animals themselves are used as

[1] The thyroidectomized animals were kept for five months and then injected with iodotyrosine and iodoserumglobulin. Metamorphosis resulted within a period of twenty days following injections of either substance. Two partially thyroidectomized animals which had failed to transform were metamorphosed by injection of iodoserumglobulin. Injections of tyrosin, dibromtyrosin and globulin had no effect upon metamorphosis. Uhlenhuth's conclusion that only thyroid iodine (iodine which has undergone transformation within the thyroid gland) is effective in metamorphosing urodele larvæ is invalid.

experimental material) can only be obtained by using thyroidectomized animals.

Professor Henry Laurens, of the Department of Physiology, informs me that several years ago he had a similar experience with axolotls from New Mexico. He received a shipment of several dozen in the spring, but was unable to prevent them from transforming shortly after arrival in New Haven. Only one animal of the lot failed to metamorphose and was kept two years in the laboratory, attaining a length of 14.25 inches. This individual was used by the writer for thyroid transplantation work.

The marked tendency of the New Mexican and other American axolotls to metamorphose spontaneously when moved from one locality to another prevents their being used for aquarium purposes. It is an odd fact that practically the only axolotls used as aquarium material in the United States are those that have been shipped from Europe.

The European strain seems to differ from the New Mexican form in regard to spontaneous metamorphosis, because these animals are handled by practically all aquarium dealers in Germany and can be obtained for a few cents apiece. Apparently they rarely spontaneously transform according to Jensen ('20), who has worked extensively with this strain. The curious thing about the New Mexican strain is that in their native habitat they too may remain for considerable periods as larva, yet when shipped from New Mexico to New Haven promptly metamorphose regardless of size or age. One large animal of this strain obtained by Professor Laurens failed to transform and was kept in the laboratory for two years; at the end of this time it showed no indications of metamorphosis and was killed for thyroid transplantation work.

According to Gadow ('08) the strain of axolotls established in Europe came originally from the vicinity of Mexico City. The first axolotls were brought to France by Marshal Forey in 1863, and the present strain is de-

scended from these animals. Gadow also states that the axolotls of Lake Xochimilco have never been known to metamorphose in their native habitat. However, several of the descendants of the animals taken to Europe did metamorphose, so that spontaneous transformation in the Mexican strain does sometimes occur, though rarely.

In an earlier paper ('22) the writer showed that the thyroid mechanism of axolotls is filled with physiologically active hormone capable of inducing metamorphosis but that the secretion is apparently not liberated into the blood stream, hence the retention of the larval characters despite the possession of a large well-formed gland. The thyroid of a fourteen-inch axolotl several years of age was extirpated and cut into small pieces, each piece then transplanted into an immature Anuran larva. The single axolotl thyroid promptly metamorphosed five such tadpoles within fourteen days, whereas left intact within the axolotl's body it was quite incapable of inducing transformation.

This same experiment was repeated upon thyroidectomized and hypophysectomized *Rana sylvatica* tadpoles with similar results. Small pieces of axolotl thyroid when engrafted into thyroidless and pituitaryless larvæ promptly induce metamorphosis within ten or twelve days.

It is quite clear from these experiments that axolotl neoteny is due to retention of the thyroid hormone within the gland vesicles. Under normal conditions and in its native habitat, the releasing mechanism apparently fails to act, but when the animals are shipped from one place to another and subjected to new environmental conditions metamorphosis promptly ensues. In the New Mexican strain slight stimulation is sufficient to initiate metamorphosis, but in the European and Mexican forms very powerful stimulation is needed to overcome the thyroid inhibition and release the secretion. In the European strain the following agents have been used successfully for inducing metamorphosis: thyroid feeding (Laufber-

ger '13), salicylic acid injections (Kaufman '18), iodine and iodoform injections (Hrischler '18–'19), organic iodine feeding—iodothyrosine, also injections of iodocasein, iodoserumglobulin and iodoserumalbumin (Jensen '21); and of course Marie von Chauvin's experiments are well known.

It is evident that the peculiar thyroid inhibition causing neoteny in axolotl is due to genetic factors and that the condition is hereditarily transmitted. It is interesting to note that in axolotl we have one of the best examples of hereditary transmission of an endocrine defect known. Attempts to explain neoteny by assuming that environmental agencies such as cold, altitude and the like are the chief causative factors are too crude to be seriously considered and for this reason—the aquarium dealers of Europe breed their animals as larvæ and the young grow up as axolotls, the matter of cold or altitude not entering into the question. As was previously mentioned, the European strain arose from a few animals taken to France in 1863.

Then, too, both Professor Laurens and myself received our animals from Albuquerque, New Mexico, where they breed. The animals were old when captured. The temperature of the pools in the vicinity of the city can not be very low even in winter—not nearly so cold as those of the middle western states, northern New York, Ohio, or Wisconsin—and axolotls have never been reported as occurring in these states so far as the writer is aware.

The *Amblystoma tigrinum* resulting from the metamorphosis of the axolotls during my experiments were placed in certain pools in the vicinity of New Haven where other species of *Amblystoma* are known to breed. The animals are full grown and should breed next spring (1923). By following the life history of the larvæ it is hoped that some new light may be shed upon the obscure and much debated problem of the relation of neoteny to environment.

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SHORTER ARTICLES AND DISCUSSION

MORE EYELESS CLADOCERA

Just before a note appeared in *Science* (Vol. 53, pp. 462–463, May 13, 1921) concerning an eyeless cladoceran individual (a *Simocephalus exspinosus*), two additional eyeless daphnids occurred in another species of the experimental stock at the Station for Experimental Evolution. These were among offspring of some *Moina rectirostris* which were being subjected to crowding in a sex-control experiment (10 mothers in each 130 c.c. wide-mouthed bottle containing about 75 c.c. of culture medium). While these two eyeless young were released on successive days and possibly in separate bottles, they were in bottles which belonged to the same series and received the same treatment.

The precise identity of the mother of neither eyeless young could be determined (since there were 10 mothers producing parthenogenetic young in each bottle), but it is certain that the mothers were normal-eyed and were sisters, or came from mothers which were sisters. All of the mothers' collaterals, which were examined, approximately 250, had normal eyes. 302 other young, produced by the 10 mothers in the bottle in which the second of these eyeless appeared, were normal. In all about 5,953 young were microscopically examined—a few of which were presumably sisters of the eyeless individuals and the others of which were young from sisters of the mothers of the eyeless individuals. All were normal-eyed.

One of these eyeless individuals produced 5 broods, containing in all 66 young, all normals. The other produced 4 broods, containing 38 individuals, all normals. 841 offspring of daughters of the one eyeless, and 412 offspring of daughters of the other eyeless were found to have normal eyes. All examined among the collaterals of the eyeless individuals, 5,953 in all, and 1,357 direct first and second generation descendants of the eyeless mothers themselves—a total of 7,310—were normal. Hence despite the fact that there were two eyeless individuals produced by sisters (or by individuals whose mothers were sisters), while among many thousands of Cladocera previously seen under the microscope only a single similar individual had

been found, eyelessness in these individuals was clearly *not* inherited. The lack of inheritance in these *Moina rectirostris* would have been anticipated if due regard had earlier been given to a peculiar feature of the head of these eyeless individuals. This will be discussed in a later paragraph.

The next occurrence of eyeless Cladocera was in February, 1922, when seven eyeless *Moina macrocopa* were found among 147 young of the third brood from 10 mothers in a crowded bottle. The culture water in this bottle seemed rather cloudy, an appearance known frequently to be associated with unfavorable conditions which sometimes result in death to part or all of the Cladocera in such a bottle.[1] In the present case in addition to one eyeless male and 6 eyeless females among the 67 females and 80 males in the bottle, there were other abnormals—6 or 8 with abnormal eyes (pigment reduced or eye not completely formed) and perhaps an equal number with abnormal antennæ (certain segments missing, aborted or fused with others) and one male with an abnormal eye and an abnormal antennule. Some of the eyeless individuals and some with abnormal eyes had abnormal antennæ also. Others showed abnormality in only one feature. Since these abnormals appeared in a crowded bottle (10 mothers) it is impossible to know, but they probably did not come from a single mother. Among the next brood of young from the same mothers were a few with abnormal antennæ and slightly abnormal eyes. Subsequent young were normal.

Early attention to an interesting feature of the heads of these eyeless individuals removed any temptation to anticipate inheritance of eyelessness in these cases; and, as expected, all the numerous young examined from these eyeless individuals (and from the other abnormals as well) were normal. Since in these cases eyelessness was not hereditary some developmental accident would seem probably responsible for its occurrence. Indeed, it seems fairly evident, in view of the occurrence of other abnormalities in the same and other similar culture bottles, that these abnormalities were related to some unfavorable fac-

[1] In other cases such conditions of the culture medium were associated with pigmentless eyes in some of the newly released young. However, the pigment develops to its full amount in from one to five days after the young animals are released from the mother's brood chamber. Newly released young from the formerly pigmentless-eyed individuals have normally pigmented eyes from the first.

tor or factors in the environment, although nothing definite is known as to what these factors were.

A peculiar structural feature of the heads of the young eyeless individuals suggested the possible manner in which eyelessness came about in these cases. When young, the seven eyeless *Moina macrocopa* had on the anterior head margin a small nodule or excrescence which, though not so conspicuous at later stages, yet in most cases persisted through several moults. In each of these eyeless individuals the optic ganglion was reduced or lacking, and the margin of the head was readjusted to compensate for the reduced and missing organs. Substantially the same structural conditions were found with the two eyeless *Moina rectirostris,* absence or reduction of optic ganglia, the shortening of the head margin and the occurrence of a small bit of apparently necrotic material attached to the front of the head.[2]

It seems possible that this apparent exudate on the heads of the eyeless individuals really represented an aborted or necrotic portion of the embryo which included the primordium of the missing parts.[3]

The fourth occurrence of eyeless Cladocera (the eleventh eyeless individual seen) was June 26 in a crowded bottle of *Moina macrocopa.* In addition to the lack of eye and of optic ganglion, the brain proper was reduced in size. This animal was not examined until mature and an excrescence on the head, if present in the young animal, had by that time disappeared. This individual swam in small circles, although its swimming organs appeared entirely normal. It died after producing two broods (10 females and 12 males) of normal young.

The occurrences of eyeless Cladocera have included three species, eleven individuals and four different time periods. The last three occurrences, and probably the first one, were in crowded bottles, suggesting environmental factors as causative

[2] That this material was intimately associated with the head structures and really a part of the animal is attested by the fact that it persisted through ecdysis, whereas any material merely adhering to the external surface of the exoskeleton would be eliminated by ecdysis.

[3] A somewhat similar appearance in larvæ arising from centrifuged eggs of *Ambystoma punctatum* was presumably correlated with failure of development of the anterior part of the head. (Banta, A. M., and Gortner, R. A., "Accessory Appendages and Other Abnormalities Produced in Amphibian Larvæ through the Action of Centrifugal Force," *Jour. Exp. Zool., 18:* 433–446, pls. 1–3. 1915.)

agents. Those which lived to produce young gave rise exclusively to normal young, indicating that genetic changes were not responsible for the abnormal heads. However, in view of the known inheritance of eyelessness in cave arthropods and vertebrates and in *Drosophila melanogaster,* it seems of interest to examine each case of profound eye modification in crustaceans and elsewhere to gain information on the origin and inheritance of any possible mutation of this character.[4]

Arthur M. Banta
L. A. Brown
Station for Experimental Evolution
Cold Spring Harbor, L. I.

CROSSING-OVER INVOLVING THREE SEX-LINKED GENES IN CHICKENS

In the course of the last year several crosses of chickens carried out at the genetics station at Anikovo (near Moscow) have made it possible to observe crossing-over in this form. The genes "suke," "tuge" and "trage" were studied. The first, suke, retards the development of feathering in the chicks, so that at the age of 1 to 1.5 months they have very small tails. The development of the wings, too, is very slow. The genes trage and tuge together cause the well-known Plymouth Rock markings, trage causing the crossbarring, and tuge (not very visible in Plymouth Rocks, where it causes the contrasts in the markings) is the same gene as silver coloring, which was first reported by Hagedoorn in the Assendelver chickens. Later (1912) Davenport observed it in the cross of Dark Brahma × Brown Leghorn, where, however, on account of the absence of several other genes, tuge has very little expression—only as a whitish edge on the feathers.

The genes suke, tuge and trage are all present together in the Plymouth Rocks. The Russian Orloff chickens have none of these genes, a condition which may be expressed as asuke-atuge-atrage. All these genes are sex-linked, and therefore are transmitted with complete linkage from mother to son. The cross

[4] Since this manuscript went to the printer two more eyeless *Moina macrocopa* were found in a crowded bottle. These two with the last one mentioned above were the only eyeless occurring among approximately 33,000 individuals microscopically examined (in sex-control experiments) during three months. The facts, that of these three two occurred in the same bottle and that the character is not inherited, again indicate clearly enough that external, not internal, factors are responsible.

Orloff male × Plymouth Rock female gives cocks closely resembling the true Plymouth Rock, that is, crossbarred with slow feathering development. All the hens, however, are black (since in the Plymouth Rock there is also a gene for melanism, "tifa," which is not sex-linked), and they develop feathers quickly.

♂: asuke atuge atrage atife × ♀: suke tuge trage tifa

F_1 ♂: suke tuge trage tifa ♀: asuke atuge atrage tifa

In F_2 the coupling between suke, tuge and trage becomes broken, and different new combinations are to be observed in rather large numbers. More often the forms asuke-tuge-trage are obtained, colored like Plymouth Rock, but with quick development of feathering (among these there are also cocks), and conversely suke-atuge-atrage, with slow feathering, but black (when tifa is present). In one case a suke-tuge-atrage chick appeared, with slow feathering and silvery, but not crossbarred.

In the light of the Morgan theory these facts can be explained by regarding the genes suke, tuge and trage as being in a sex chromosome which cannot give crossing-over in the heterozygous sex (female). But when the same chromosome is transmitted to the F_1 male, it undergoes crossing-over with its partner, which occurs most often in the space between suke on the one side and tuge-trage on the other. Crossing-over between suke-tuge on the one side and trage on the other occurs less often, wherefore the arrangement of the genes in the F_1 may be represented as follows:

suke......................tuge..........trage

However, the counts of chicks which have so far been obtained in F_2 are not yet large enough to ascertain definitely the order of the genes, and therefore still less the exact distances.

A. S. SEREBROVSKY

INSTITUTE OF EXPERIMENTAL BIOLOGY,
41 SIVTSEV VRAZHEK,
MOSCOW, August 21, 1922

[Crossing-over between "suke" (barring) and "tuge" (silvery) has also been announced by Goodale (1917) and by Haldane (1921), in the papers listed below, which were not available to the above author.

Goodale, H. D. 1917. Crossing-over in the Sex Chromosome of the Male Fowl. *Science*, N. S., Vol. 46, p. 213.

Haldane, J. B. S. 1921. Linkage in Poultry. *Science*, N. S., Vol. 54, p. 663.

Note of Transmitter, H. J. Muller.]

A FOURTH ALLELOMORPH IN THE ALBINO SERIES IN MICE[1]

In recording the occurrence of a new mutant gene in the house mouse, allelomorphic to color and albinism, Detlefsen ('21)[2] described a very dilute, wild form in which the hair showed traces of a light brownish tinge with a suggestion of sootiness, and the eyes were somewhat less heavily pigmented than in the wild type. This general form of pigment reduction is also characteristic of other color allelomorphs; for in the case of the ruby-eyed rat, the ruby-eyed guinea-pig and the chinchilla rabbit (Castle '21),[3] the yellow pigment is very greatly reduced or even obliterated, while the darker pigments (black or brown) are at least slightly modified. The mutant mouse, however, showed a far greater pigment reduction than either the rat, guinea-pig or rabbit mutants. Breeding tests demonstrated that this dilute mouse mutant was a color-albino allelomorph, and in this respect resembled the ruby-eyed rat and guinea pig genetically (the chinchilla rabbit had not been recorded at that time), but Dr. Detlefsen pointed out that "it is hardly safe to insist that these mutations are identical. . . . We are also unable to prove that they are different, for the genes may be identical but simply give different somatic effects, since the residual inheritance can not be the same." He also suggested that the discovery of a new dilute type of mouse (which he was seeking at that time), more like the rat or guinea pig in its somatic appearances as well as in its genetic behavior, would give us more assurance that his extreme dilute mouse mutant was not the homolog of the ruby-eyed rat or guinea pig. Unusual as it may seem, I had discovered exactly such a new dilute mutant mouse in January, 1919. By comparing it with Dr. Detlefsen's set of rodent skins and by testing it in appropriate matings, I recognized its genetic significance just before his paper appeared in print.

The discovery of this new mutant mouse enables us to say at once that the extreme dilute mutant was not the homolog of the ruby-eyed rat or guinea pig or the chinchilla rabbit, and supports Dr. Detlefsen's position in hesitating to homologize

[1] Paper No. 22 from the Genetics Laboratory, College of Agriculture, University of Illinois.

[2] Detlefsen, J. A., 1921, AMER. NAT., Vol. 55, p. 469.

[3] Castle, W. E., 1921, *Science*, N. S., Vol. LIII, p. 387.

his mutant with these forms. Dr. Detlefsen's form is evidently lower in the scale running from color to complete albinism in very much the same way that the Himalayan form is nearer to the albino than is the chinchilla rabbit.

The new mutant was procured from a fancier who had been breeding it for some time. It resembles the ruby-eyed guinea pig, ruby-eyed rat and the ruby-eyed or chinchilla rabbit (Castle '21)[4] in the degree of pigment reduction in the hair, but the eyes are apparently darker than those of the rat and guinea pig. I have not had an opportunity to examine the eyes of the chinchilla rabbit. It forms one of a series of quadruple color allelomorphs in the mouse and may be designated as c^r. In a scale of dominance, the four forms probably fall into the following order: ordinary intense or wild color, C; dilute, c^r (described in this paper); extreme dilute, c^d (described by Detlefsen ('21);[5] and complete albinism, c. Wild color (C) is completely dominant to the other allelomorphs, but c^r and c^d are incompletely dominant to albinism. The cross between c^r and c^d has not yet been made, but the heterozygote (c^rc^d) will probably be found to give an intermediate shade.

The black agouti type of the homozygous mutant ($AABBc^rc^r$) possesses black pigment which is reduced to a very dark dull slate-color, while yellow is greatly reduced and appears about intermediate between white and the normal yellow of the wild type. In the non-agouti type of the homozygous mutant ($aaBBc^rc^r$), which can be distinguished readily from the agouti form, the black pigment is also reduced to a very dark dull slate-color, but perhaps darker than in the agouti type.

When the black agouti type of the mutant is heterozygous for albinism ($AABBc^rc$), black pigment is reduced to a brownish shade and yellow is practically reduced to white. In the non-agouti type of the heterozygous mutant ($aaBBc^rc$), black is reduced to a dull brown, a little lighter than the ordinary fancier's chocolate type. The heterozygous mutants, mated interse, produce the homozygous type, the heterozygous type and albinos in the ratio of 1 : 2 : 1.

I have not yet identified the mutant without black pigment that is in the cinnamon or brown class.

H. W. FELDMAN

[4] *Loc. cit.*

[5] *Loc. cit.*

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